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A Comparative Histological Study of the Anterior Hypophysis and the Ovaries of Two Strains of Rats, One of Which Is Characterized by a High Incidence of Mammary Fibroadenoma*

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For the past few years we have been studying a strain (Albany) of rats in which the incidence of spontaneous mammary fibroadenoma is relatively high (1, 3). Because of the unusual incidence, we have examined these rats for other peculiarities that might be related to the appearance of the new growths or might suggest some reason for their development. Apart from the frequent occurrence of mammary tumors the most obvious peculiarity of this strain is its low fertility, which has often made it difficult to maintain certain sublines. Investigation of this condition has revealed that the Albany rats present certain endocrine abnormalities that strongly suggest a constitutional hypofunction of the anterior pituitary gland. These include a complete or partial failure of ovulation and subsequent follicular atresia in many rats (20) that result in abnormalities of the estrous cycle, a high percentage of fetal resorption accompanied by vaginal hemorrhage during pregnancy (2), a certain degree of testicular degeneration in the males, and a considerable retardation of growth rate (19). If an inherent anterior pituitary dysfunction were present in this strain, it seems logical that certain structural abnormalities might exist in the anterior hypophysis that would form a structural basis for the functional abnormalities noted above. A histological study of the anterior hypophyses of the Albany strain of rats was therefore made and the glands were compared with those of a control strain (Vanderbilt) in which growth and reproduction are excellent and the incidence of benign mammary tumors is very low. In this communication, our findings are presented.

We have recently reported (23) that advancing age in female rats of the Vanderbilt strain is associated with structural changes in the anterior hypophysis; these will be described presently. In comparing the anterior lobes of these two strains it was important that this age factor be considered. Therefore the anterior hypophyses from rats of both strains at all periods of the life span were studied, the animals falling into the following age groups: 1, 3, 6, 12, 17 to 19, 20 to 24, and 25 to 28 months. Approximately 50 per cent of the Albany rats in the age groups above 12 months had mammary tumors. In reality this study was concerned not only with a histological comparison of the anterior hypophyses from the two strains, but also with the comparative effects of advancing age on this gland in both. The number of rats used in each group is indicated in Table I. In order to eliminate the factor of the structural effects of pregnancy, the Albany rats were either virgin or sterile and the Vanderbilt rats were virgins.

Since the structure of the ovaries may be considered an accurate index of anterior lobe activity, we have also studied the ovaries of rats in all age groups of both strains and have correlated their structure with that of the anterior hypophysis. This study has also yielded further information on the relation of advancing age to the abnormal ovarian function already described in the Albany rats. These two strains have been maintained under identical conditions since 1936

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TABLE I: SUMMARY OF THE STATISTICAL ANALYSIS MADE ON THE QUANTITATIVE DATA FROM THE ANTERIOR HYPOPHYSES OF THE ALBANY AND VANDERBILT RATS

Age group	Strain of rats	Number of rats	Body weight average, gm.	Pituitary weight average, mgm.	Percentages of cells: Eosinophils	Granular basophils	Nongranular basophils	Chromophobes
1 month	Vanderbilt	28	84	3.5	M.40.9 ± 0.5 SD.3.8 ± 0.3	M. 5.0 ± 0.4 SD.2.9 ± 0.3	M. 3.8 ± 0.3 SD.2.1 ± 0.2	M.50.3 ± 0.6 SD.4.4 ± 0.4
	Albany	27	71	2.5	M.31.8 ± 0.5 SD.3.9 ± 0.4	M. 4.4 ± 0.3 SD.2.5 ± 0.2	M. 3.9 ± 0.2 SD.1.8 ± 0.2	M.59.9 ± 0.6 SD.4.6 ± 0.4
3 months	Vanderbilt	30	215	10.1	M.34.8 ± 0.5 SD.3.8 ± 0.3	M. 1.1 ± 0.1 SD.0.9 ± 0.1	M. 3.1 ± 0.1 SD.1.1 ± 0.1	M.61.0 ± 0.5 SD.3.8 ± 0.3
	Albany	31	169	8.7	M.27.9 ± 0.6 SD.4.7 ± 0.4	M. 1.7 ± 0.1 SD.0.9 ± 0.1	M. 4.9 ± 0.1 SD.1.2 ± 0.1	M.65.5 ± 0.6 SD.5.3 ± 0.5
6 months	Vanderbilt	24	252	11.7	M.30.5 ± 0.6 SD.4.5 ± 0.5	M. 2.4 ± 0.2 SD.1.3 ± 0.1	M. 3.3 ± 0.2 SD.1.1 ± 0.1	M.63.8 ± 0.7 SD.5.2 ± 0.5
	Albany	29	198	10.7	M.22.3 ± 0.6 SD.4.7 ± 0.4	M. 1.9 ± 0.2 SD.1.6 ± 0.1	M. 3.4 ± 0.2 SD.1.4 ± 0.1	M.72.3 ± 0.8 SD.6.1 ± 0.5
12 months	Vanderbilt	30	284	14.1	M.25.0 ± 0.7 SD.5.5 ± 0.5	M. 2.3 ± 0.1 SD.0.9 ± 0.1	M. 2.8 ± 0.1 SD.0.7 ± 0.1	M.69.9 ± 0.7 SD.5.9 ± 0.5
	Albany	29	219	12.6	M.17.1 ± 0.3 SD.2.6 ± 0.2	M. 1.5 ± 0.1 SD.0.8 ± 0.8	M. 3.1 ± 0.1 SD.1.1 ± 0.1	M.78.3 ± 0.4 SD.3.3 ± 0.3
17 to 19 months	Vanderbilt	20	310	15.8	M.22.7 ± 0.6 SD.3.8 ± 0.4	M. 1.8 ± 0.2 SD.1.3 ± 0.1	M. 3.3 ± 0.1 SD.1.0 ± 0.1	M.72.2 ± 0.7 SD.4.7 ± 0.5
	Albany	50	240	11.9	M.17.1 ± 0.4 SD.4.3 ± 0.3	M. 2.4 ± 0.1 SD.1.2 ± 0.1	M. 2.6 ± 0.1 SD.1.5 ± 0.1	M.77.9 ± 0.5 SD.5.1 ± 0.3
20 to 24 months	Vanderbilt	24	330	16.8	M.20.4 ± 0.4 SD.2.7 ± 0.3	M. 1.7 ± 0.1 SD.1.0 ± 0.1	M. 2.9 ± 0.1 SD.1.1 ± 0.1	M.75.0 ± 0.5 SD.3.5 ± 0.3
	Albany	41	242	12.3	M.16.5 ± 0.4 SD.3.6 ± 0.3	M. 2.0 ± 0.1 SD.1.2 ± 0.1	M. 2.8 ± 0.1 SD.1.3 ± 0.1	M.78.7 ± 0.5 SD.4.4 ± 0.3
25 to 28 months	Vanderbilt	10	301	14.7	M.19.9 ± 0.6 SD.2.8 ± 0.4	M. 1.8 ± 0.2 SD.0.8 ± 0.1	M. 2.4 ± 0.3 SD.1.4 ± 0.2	M.75.9 ± 0.5 SD.2.0 ± 0.3
	Albany	21	225	12.3	M.16.1 ± 0.6 SD.3.9 ± 0.4	M. 2.0 ± 0.2 SD.1.6 ± 0.2	M. 2.8 ± 0.2 SD.1.4 ± 0.1	M.79.1 ± 0.8 SD.5.4 ± 0.6
Significance ratio * of difference between means	1 month Vanderbilt and Albany				12.6	1.2	0.27	11.4
	3 months Vanderbilt and Albany				9.3	3.7	9.0	5.6
	6 months Vanderbilt and Albany				9.5	1.8	0.4	8.0
	12 months Vanderbilt and Albany				10.3	5.3	1.8	9.8
	17 to 19 months Vanderbilt and Albany				7.7	2.7	3.1	6.6
	20 to 24 months Vanderbilt and Albany				6.9	1.3	0.4	5.2
	25 to 28 months Vanderbilt and Albany				4.4	0.7	1.1	3.4

* Significance ratio was calculated by the formula

$$\frac{\text{Mean 1} - \text{Mean 2}}{\sqrt{(\text{P.E. Mean 1})^2 + (\text{P.E. Mean 2})^2}}$$

When the ratio equals 3 or over, it is significant.

and the structural differences to be described in their anterior hypophyses and ovaries must be considered as constitutional in nature and not due to environmental factors.

At autopsy, body and organ weights were obtained. The hypophyses were fixed in Regaud's fluid and prepared for examination by our standard method (4). They were studied histologically, cell counts were made, and from these counts the percentages of the various cell types were calculated. The resulting quantitative data were analyzed statistically (Table I). The reproductive organs were fixed in Bouin's fluid and stained with hematoxylin and eosin. The ovaries were cut serially and studied by quantitative methods to be described presently. Finally, representative sections of the uteri and vaginae were examined.

span of the rats. Since these differences were found even in the 1 month old rats, it is considered likely that they were established in the fetal pituitary.

Further analysis of Table I and Fig. 1 indicates that quantitative changes, which could be correlated with advancing age, occurred in the anterior lobes of both strains. Such changes in the anterior hypophyses of the Vanderbilt rats have already been described in detail (23); the data in Table I and Fig. 1 pertaining to this strain are taken from this previous study. Briefly, it was found that advancing age in virgin Vanderbilt rats was associated with a progressive and a statistically significant decrease in the relative number of the eosinophils and that this decrease was most rapid early in the life span. Inversely, the relative number of the chromophobes was increased to virtu-

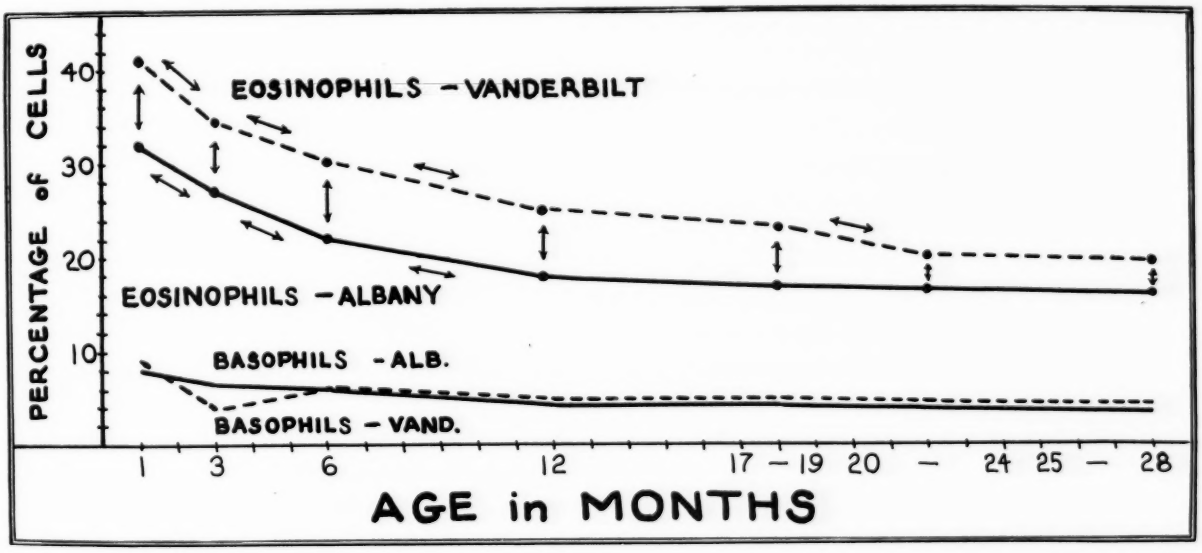

FIG. 1.—Percentages of eosinophils and basophils in the anterior hypophysis of Albany and Vanderbilt rats, and the response of these cells to advancing age. The arrows between the various points on the curves for the eosinophils indicate that the differences in the levels of the cells at the two points are statistically significant.

OBSERVATIONS

Comparative Study of the Anterior Hypophysis

Analysis of the cell counts revealed that in all the age groups considered the anterior hypophyses of the Albany strain were characterized by significantly lower percentages of eosinophils than were found in the glands of the Vanderbilt strain in the same age groups, while the relative number of the chromophobes was significantly higher (Table I and Fig. 1). In contrast to these findings, no significant or constant difference was found in the relative number of the basophils in the two strains in any age group (Table I and Fig. 1). These findings indicate, therefore, that there were definite quantitative structural differences involving the eosinophils and the chromophobes in the anterior hypophyses of the Albany and Vanderbilt strains, and that these differences were present throughout the life

ally the same degree that the eosinophils were decreased. Quantitative age changes in the basophils were not pronounced. Granular basophils were significantly more abundant in 1 month old rats than in any of the mature rats, but beyond the age of 3 months the percentages of the basophils remained practically stationary.

Age changes essentially similar in nature to those that occurred in the Vanderbilt rats were found in the anterior hypophyses of the Albany rats (Table I and Fig. 1), although in all age groups the percentages of these cells were significantly lower than they were in the Vanderbilt rats. The decrease in the relative number of the eosinophils that was associated with advancing age in the Albany rats was rapid early in the life span and was complete in the 12 month old rats. As a result, the levels of these cells remained practically stationary in the Albany rats older than

12 months. In the Vanderbilt rats there was also a pronounced decrease in the percentages of the eosinophils early in life, but this decrease was not completed at 12 months of age; in fact these cells continued to decrease in relative number, at a slower rate, throughout the remainder of the life span. In the Albany rats, therefore, practically all the total decrease destined to occur in the percentages of the eosinophils during the life span had already occurred at 12 months of age, while in the Vanderbilt rats approximately 75 per cent of this decrease had occurred at the same age. This variation in the response of the anterior lobes of the two strains to advancing age, and the significantly lower levels of the eosinophils in the anterior lobe of the Albany rats in all age groups constitute the chief, and we think the most significant, structural differences between the hypophyses of the two strains.

The effects of advancing age on the basophils were not pronounced. In the 1 month old Albany rats granular basophils were significantly more abundant than in the adult rats (Table I). In the mature rats both granular and nongranular basophils were found, but their number was relatively constant throughout life. In Fig. 1 granular and nongranular basophils have been combined. With the exception of the high levels in the 1 month old rats no quantitative changes occurred with increasing age. These findings are identical for those of the Vanderbilt rats (Fig. 1).

In addition to the strictly quantitative changes mentioned above, certain other structural alterations that progressed with age occurred in the anterior lobes of the rats of both strains. These included a decrease in the number of mitoses; the appearance and increase of basophilic vacuolization; colloid degeneration of the anterior lobe cells, and deposition of acidophilic colloid between the cells and in the residual cleft; the development of adenomatous changes; and the occurrence of cytological alterations in the anterior lobe cells.

The degree of mitotic proliferation was approximately the same in the two strains, mitoses having been found in both the eosinophils and the chromophobes. In both strains mitoses were most frequently found in the immature rats, were progressively less numerous in rats 3 and 6 months of age, and were found infrequently in those beyond this age.

Vacuolated basophils occur in the anterior pituitary gland of the rat in a variety of circumstances. They are most numerous in castrated and thyroidectomized rats, where they are known as castration or thyroidectomy cells; there is some question, however, concerning the identical nature of these two types; the pertinent literature has been given by Severinghaus (12). Destruction of the germinal epithelium in male rats, such as that found in cryptorchid and vitamin E deficient male rats, also leads to the appearance of a smaller number of vacuolated basophils in the anterior lobe (5, 8, 14), while a few of these cells are found in the anterior lobes of normal male rats (5, 7).

It has already been reported that occasional vacuolated basophils are found in the anterior hypophyses of female Vanderbilt rats (23). They were first noted in approximately half the anterior lobes from the 3 month old rats, although the average number of these cells per section was only 0.4. As age advanced, vacuolated basophils became progressively more numerous and were found in a greater percentage of the glands studied. In the rats 20 to 24 months of age the average number of vacuolated basophils per section was 8.9, and they were present in all glands. Vacuolated basophils were found in the anterior lobes of the Albany rats also, and their number and response to advancing age were almost identical with that of the Vanderbilt rats although they were slightly more numerous at 17 months of age and more. The exact significance of basophilic vacuolization is not known at present. It has been suggested that it is a degenerative process in the basophil (11), a view with which we agree. Regardless of the significance of these cells, their appearance and subsequent increase in number is a manifestation of advancing age in both the Albany and Vanderbilt rats.

A degenerative process in which the anterior lobe cells undergo transformation into small masses of acidophilic colloid has already been described in the Vanderbilt rats (23). Such colloid degeneration appeared in these animals at approximately 3 months of age, and rapidly increased in intensity. It reached a maximum in the 6 and 12 month rats, and then remained more or less constant throughout the remainder of the life span. As a result of this cellular degeneration, masses of intercellular acidophilic colloid appeared throughout the gland. The same process of colloid degeneration and deposition of intercellular colloid occurred in the anterior hypophyses of the Albany rats, but it appeared earlier and the reaction was more intense. In the Vanderbilt strain the first evidence of colloid degeneration of the anterior lobe cells appeared in the 3 month old rats, while in the Albany strain the change was found in slight degree in approximately one-half of the 1 month old rats. In the succeeding age groups the process was almost invariably more intense and more widespread in the anterior lobes of the Albany rats.

Acidophilic colloid, which was found in the residual cleft of both strains, also appeared earlier and was considerably more abundant in the Albany strain, appearing first in the 1 month old rats, while in the Vanderbilt rats it was not observed, with the exception of traces in 3 rats, until the age of 3 months. In both strains the amounts of cleft colloid reached a maximum

at 6 and 12 months of age, and there was no further increase in the older rats.

Certain cytological changes occurred in the anterior lobe cells of both strains that were definitely correlated with the structural, and presumably the functional, condition of the ovaries. However, since the reproductive system undergoes such pronounced alterations during the various periods of life, the cytological changes were also correlated in a general way with advancing age. Their analysis was largely based on the studies of Severinghaus (11) and of Wolfe and Brown (22), who have associated enlargement of the Golgi apparatus with elaboration of secretion by the anterior lobe cells and degranulation of the cell with release of secretion. In the anterior lobes of the immature rats of both strains practically all the eosinophils and a majority of the basophils were well filled with granules and showed little evidence of loss of secretion. In the mature rats of all ages in which evidence of ovarian activity was present, all stages of degranulation in the eosinophils were noted, and in many there was hypertrophy of the Golgi apparatus. Both granulated and degranulated basophils were seen; the numbers of each presumably depended on the phase of estrous cycle in which the rat was killed. Likewise, chromophobes exhibiting various degrees of hypertrophy of the Golgi apparatus were seen. In those old rats that showed a decrease in ovarian activity there was a corresponding decrease of secretory phenomena in the anterior pituitary cells; and in the few in which there was decided atrophy of the ovaries the eosinophils of the hypophysis were small and showed practically no evidence of degranulation, and the number of granular basophils tended to be increased slightly. Since regression of the ovaries generally occurred earlier in the Albany rats, as will be apparent later in this paper, there was a somewhat earlier regression of secretory changes in the anterior lobes of rats of this strain.

It has previously been reported that spontaneous adenomatous changes occur rather frequently in the anterior hypophyses of old rats of the Vanderbilt strain, both male and female, and a description of the lesions was given (18). Similar findings were later reported by Saxton (9) in rats of the Yale strain. Such adenomatous changes were found in the anterior pituitary glands of old rats of both the Albany and Vanderbilt strains considered in this paper. In the Vanderbilt rats the lesions were found in the anterior lobes of 27 per cent of all those 17 months of age and over, while the incidence in the Albany rats of the same age was only 11.4 per cent. This finding makes it quite evident that the factor or factors in the Albany strain determining the appearance of mammary tumors do not simultaneously increase the incidence of anterior lobe adenomas.

The findings recorded in this paper, as well as those previously made in the Vanderbilt strain, indicate that advancing age in the rats of both strains is associated with definite structural alterations in the anterior hypophysis that qualitatively are the same in the two strains. These findings further indicate, however, that most of these alterations occur earlier and to a more intense degree in Albany rats.

Comparative Study of the Ovaries

In the histological comparison of the ovaries of the two strains quantitative methods were used whenever possible. With the exceptions noted below all follicles, both normal and atretic, and corpora lutea were counted and are listed in Table II and Figs. 2, 3, and 4 as the average number per ovary. The two greatest diameters of these same structures were measured in millimeters and the average was taken as an index of their size. Follicles with an average diameter of less than 0.3 mm. and corpora lutea with an average diameter of less than 0.5 mm. were not counted. In addition, a general histological examination was made of each ovary. The ovaries of rats in all age groups except those of the rats 1 month old were studied. In every group definite structural differences between the ovaries of the Albany and Vanderbilt rats were found. In general, the response of the ovaries of the Albany rats to advancing age was the same as in the Vanderbilt rats but the changes appeared earlier and were more widespread and more intense.

Reference to Table II and Fig. 2 reveals that in the Vanderbilt rats normal follicles were most abundant in the ovaries of 3 month old rats, and that as age increased there was a slow and progressive decrease so that the smallest number of follicles per ovary was reached in the 25 to 28 month age group. In the 3 month old Albany rats normal follicles were considerably more abundant than in Vanderbilt rats of the same age (Table II and Fig. 2), but at 6 months they were so much diminished in number that they were less numerous than in the ovaries of the Vanderbilt controls. As age advanced the number of normal follicles in the Albany rats continued to decline much more rapidly than in those of the Vanderbilt strain, so that the minimum number per ovary was reached in the 17 to 19 month age group. At this age the ovaries of the Albany rats contained only about one-half the number of normal follicles found in those of the Vanderbilt rats of the same age. From this age onward until 25 to 28 months the number of normal follicles in the Albany rats remained practically constant. Thus in both the Albany and Vanderbilt strains a diminution in the number of normal ovarian follicles was associated with advancing age, but this decline

was so rapid in the Albany rats that the ovaries of 17 to 19 month old animals contained no more normal follicles than were found in Vanderbilt rats approximately 6 months older.

The relation of follicular atresia in the ovaries of the two strains to advancing age was more difficult to analyze. In the Vanderbilt rats the number of atretic follicles was initially high in the 3 month old rats, but declined greatly in the 6 month old animals and then remained approximately stationary through the age period of 17 to 19 months, after which their number increased considerably (Table II and Fig. 2). Thus throughout the most active portion of the reproductive period atretic follicles were less numerous than normal ones, but associated with waning ovarian activity in rats beyond 20 months of age, follicular atresia was increased and atretic follicles outnumbered the normal.

In the Albany strain the number of atretic follicles was greatest in the 3 month old rats; this number was greater than that found in the Vanderbilt rats of the same age (Table II and Fig. 2). Throughout life, until the age of 17 to 19 months, their numbers declined but beyond the age of 3 months atretic follicles were always more abundant than normal follicles in the ovaries of the Albany rats. Thus, with the exception of a period early in life, the ratio of atretic to normal follicles was consistently greater in the Albany strain than in the Vanderbilt (Table II).

Although the presence of atretic follicles indicates that the animal lacked the capacity to maintain the structure of the follicle in a normal condition, their presence indicates also the capacity at least to initiate follicular growth and to maintain it to a limited degree. Therefore, the numbers of normal and atretic follicles in each ovary were added together and the total number of follicles taken as an index of the capacity of the rat to initiate follicular growth in the ovary. In the Albany rats 3 months old the total number of follicles was much greater than in the Vanderbilt rats, it dropped sharply in the 6 month old rats, and fell below the Vanderbilt level at the age of 12 months (Fig. 3). Although the total number of follicles in the ovaries of the Vanderbilt rats was initially lower than the number found in animals of the Albany strain, the former animals exhibited an increased capacity to maintain a fairly constant rate of follicular growth throughout life. Thus the Albany rats were distinctly inferior to those of the Vanderbilt strain in their capacity to maintain the normal structure of the graafian follicles and the rate of follicular growth. The reason for the initial high number of follicles in the young Albany rats is unknown.

The most pronounced, and probably the most significant, structural difference in the ovaries of the Vanderbilt and the Albany rats was the decreased number of corpora lutea found in the ovaries of the latter (Table II and Fig. 4). This reduction in the number of corpora lutea in the Albany strain was direct evidence of the failure of ovulation already mentioned. In the Vanderbilt rats the rate of production of corpora lutea was maintained at a fairly constant level through the age group of 17 to 19 months; a sharp decrease in the average number of corpora lutea per ovary then occurred (Table II). The situation in the Albany strain was in pronounced contrast; in the rats 3 months of age corpora lutea were slightly more numerous than in Vanderbilt rats of the same age, but as age increased the numbers of corpora lutea declined rapidly and continued to do so throughout

TABLE II: SUMMARY OF QUANTITATIVE DATA SHOWING REACTION OF THE OVARIES OF THE VANDERBILT AND ALBANY RATS TO ADVANCING AGE

Age, months	3		6		12		17 to 19		20 to 24		25 to 28	
Strain	Vanderbilt	Albany	Vanderbilt	Albany	Vanderbilt	Albany	Vanderbilt	Albany	Vanderbilt	Albany	Vanderbilt	Albany
Number of normal follicles per ovary	12.1	19.6	10.4	9.9	9.7	6.9	7.5	3.7	5.6	3.8	2.9	2.7
Number of atretic follicles per ovary	13.1	16.9	7	11.9	6.0	8.3	6.9	5.2	9.3	7.6	8.7	4.4
Ovaries with corpora lutea, per cent	100	100	100	100	100	71.2	90.2	66.8	90.0	58.6	89.0	50.0
Number of corpora lutea per ovary	17	18.3	19.8	17	19.1	11	18.3	9.2	10.4	7.3	9.9	5.4
Ovaries with wheel cells, per cent	None	None	None	26.7	6.8	47.6	47.8	92.8	70.0	72.5	78.0	80.0
Ovaries with cystic follicles, per cent	None	None	None	None	5.2	28.5	9.8	11.2	25.0	27.5	27.7	15.0
Ratio A. F.* to N. F.†	1.1–1	0.9–1	0.6–1	1.2–1	0.6–1	1.2–1	0.9–1	1.4–1	1.9–1	2–1	2.4–1	1.4–1

* Atretic follicles.
† Normal follicles.

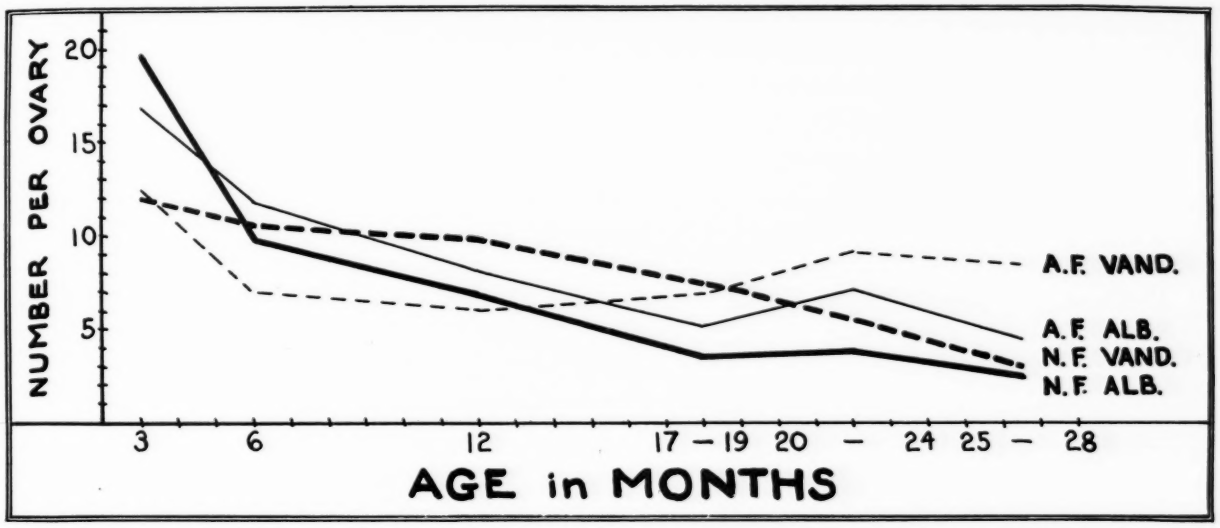

FIG. 2.—Average numbers of normal follicles (N. F.) and atretic follicles (A.F.) in the ovaries of Albany and Vanderbilt rats and their response to advancing age.

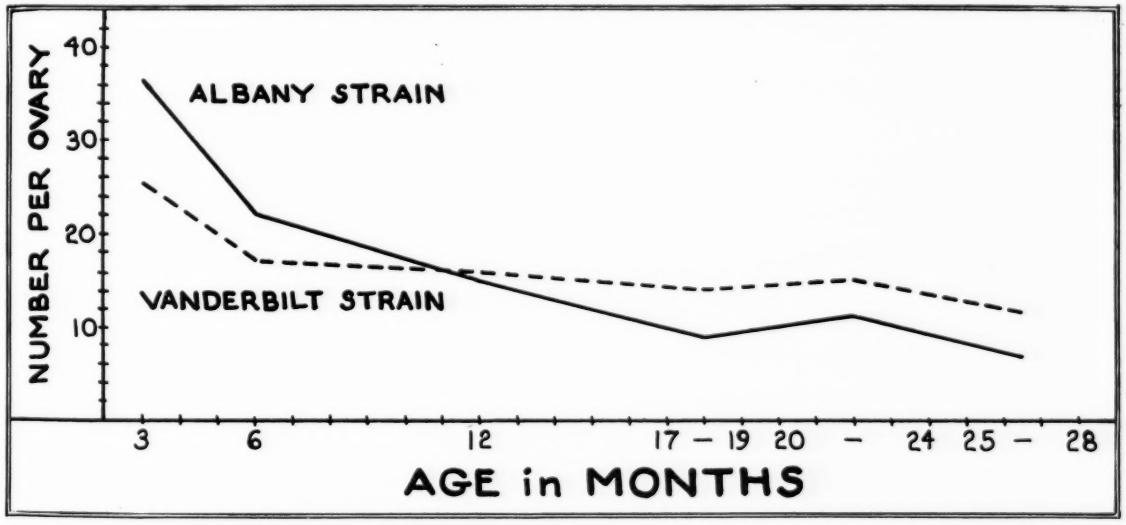

FIG. 3.—Averages of the total numbers of follicles (normal plus atretic) in the ovaries of Albany and Vanderbilt rats and their response to advancing age.

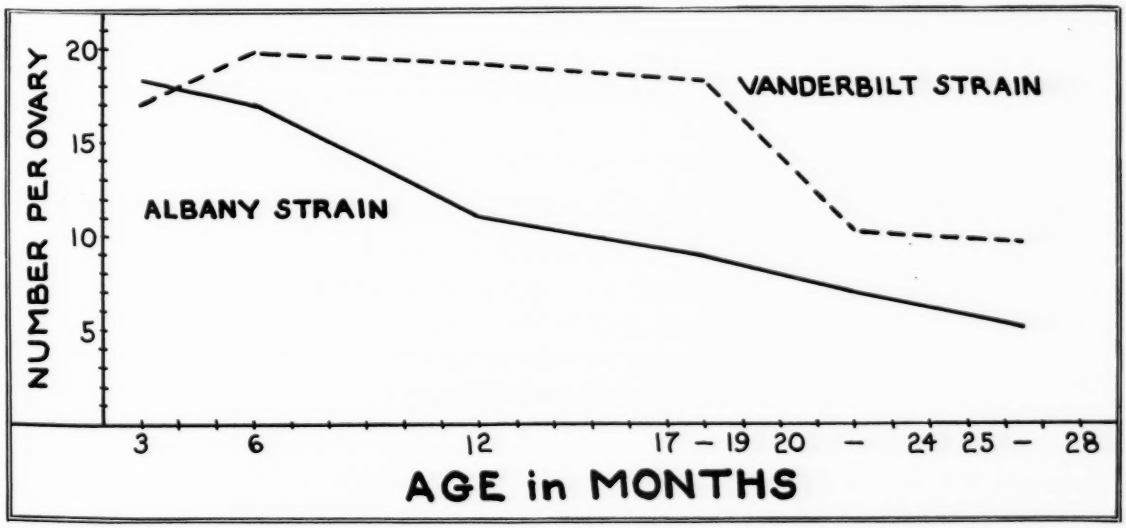

FIG. 4.—Average numbers of corpora lutea in the ovaries of Albany and Vanderbilt rats, and their response to advancing age.

the remainder of the life span. This finding can be considered as direct evidence of a gradual failure of ovulation in the Albany rats that began rather early (Table II and Fig. 4). Furthermore, the ovaries of the Albany rats were characterized by other irregularities of luteinization. Direct luteinization was noted frequently and often there was also evidence of faulty luteinization; the lutein cells were decreased in number and much of the body was made up of a loose vascular stroma. Reference to Table II will show that as age advanced ovaries were observed in both strains in which there was a complete absence of corpora lutea, indicating a total failure of ovulation. However, this situation appeared earlier and was much more pronounced in the Albany strain. As an example, 100 per cent of the Vanderbilt rats in the age group of 12 months contained corpora lutea in their ovaries, but in the Albany strain the ovaries of only 71.2 per cent of the rats of this age contained corpora lutea (Table II).

It has already been pointed out (20) that wheel or deficiency cells, first described by Selye and his associates (10) in the ovaries of hypophysectomized rats, appeared in the ovaries of a small percentage of the Vanderbilt rats 12 months of age and that their incidence increased as age advanced (Table II and Fig. 5). Their presence was accepted as evidence of decreasing anterior lobe stimulation of the ovaries that progressed with age. The wheel cells occurred also in the ovaries of the Albany strain but they appeared much earlier, for they were present at 6 months of age and throughout the remainder of the life span the wheel cell reaction was definitely more pronounced in the ovaries of the Albany rats.

Cystic follicles (follicles with an average diameter of 0.9 mm. or more) appeared in the ovaries of both strains at the age of 12 months, and their incidence increased as age advanced (Table II). There was no appreciable difference between the two strains in the incidence of these structures except that they were much more numerous in the Albany rats in the 12 month age group.

The comparative study of the ovaries in the two strains indicates that ovarian function is decidedly inferior in the Albany rats. These findings account for the high incidence of sterility that was noted originally in the females. It is further clear that structural changes denoting a decrease of ovarian function appear in both strains as age advances. However, these set in much earlier and are present to a much greater degree in the ovaries of the Albany rats. As a whole, rats of the Vanderbilt strain exhibit an inherent capacity to maintain relatively normal ovarian function until near the end of the second year of life. In contrast, the Albany rats lack this high capacity to maintain this essential bodily function; therefore, evidence of ovarian failure begins much earlier in the life span. Since the anterior hypophyses of the Albany strain also present certain structural abnormalities and an atypical response to advancing age, the concept that the Albany rats suffer from an inherent anterior pituitary hypofunction seems justified.

On the basis of the early regressive changes that occur in the ovaries of the Albany rats we have recently

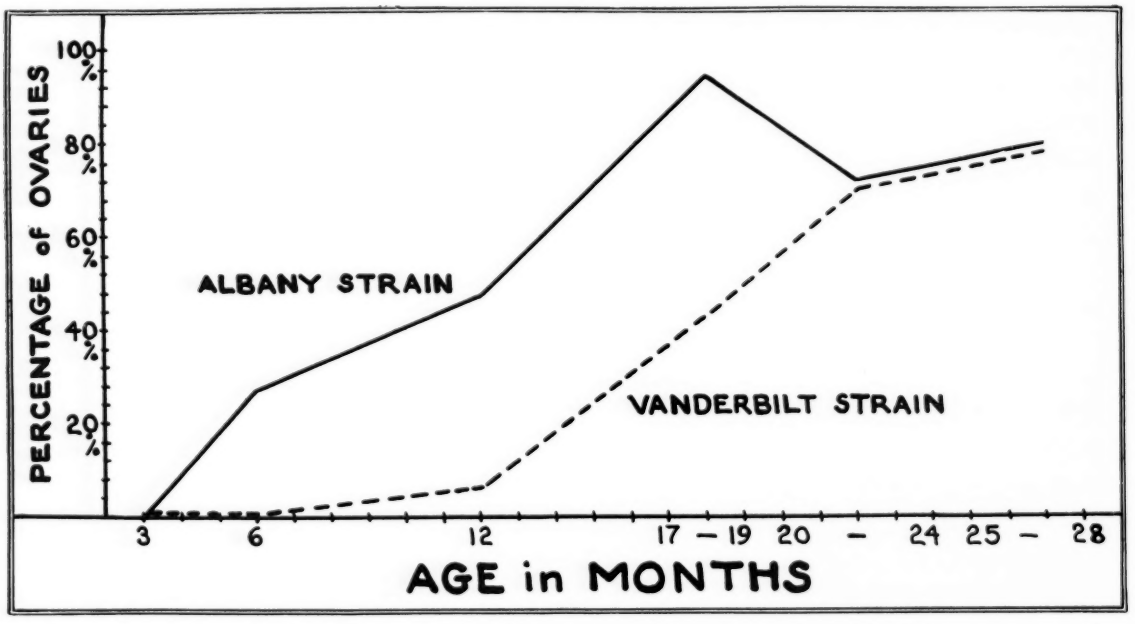

FIG. 5.—Percentages of ovaries of Albany and Vanderbilt rats containing wheel cells, and their increase as age advances.

suggested (24) that these animals show evidence of premature senescence. The present observations indicate that premature age changes occur in both the anterior hypophyses and ovaries of this strain; whether they take place in the other organs and tissues is not known at the present time. Since there is evidence of both anterior lobe hypofunction and premature aging of certain organs in this strain, it seems to us highly probable that these two abnormalities are intimately related.

Comparison of the Anterior Hypophyses and the Ovaries of the Albany Rats with and without Mammary Tumors

It has already been pointed out that the structural abnormalities in the anterior hypophyses and the ovaries of the Albany strain as a whole tend to be more pronounced in those rats that actually have mammary tumors (18). Thus the relative number of eosinophils in the anterior lobes of tumor bearers was significantly lower than in rats without tumors; inversely, the percentages of chromophobes were higher in the rats with tumors. In the present study we have compared the anterior hypophyses of Albany rats with and without mammary tumors in 3 age groups; *i.e.*, 17 to 19 months, 20 to 24 months, and 25 to 28 months. In each group the average percentages of the eosinophils were lower in the tumor animals, but the difference was great enough to be statistically significant in only the 25 to 28 month old group. The animals were then combined to form two groups, one 17 to 28 months old with mammary tumors and one of the same age without mammary tumors. These were compared with each other (Table III), and it was found that the average percentage of eosinophils was lower and the average percentage of chromophobes higher in the rats with mammary tumors. Although the differences were not great, they were sufficient to be statistically significant. The nongranular basophils were also significantly more numerous in the rats without mammary tumors.

The ovaries of the same groups of rats were also compared in the same way (Table IV). It was found that the average numbers of both corpora lutea and normal follicles were less in the rats bearing mammary tumors, but that the differences between the two groups were not quite great enough to be statistically significant. Furthermore, the numbers of atretic follicles and the incidence of wheel cells were practically the same in both.

These findings indicate, therefore, that the structural differences in the ovaries of the two groups of Albany rats were not as pronounced as those found in the anterior hypophyses. In fact, in the case of both the ovaries and the anterior lobes there was great individual variation in the rats of both groups and the differences between the two groups were never great. However, these data do seem to indicate that there is a definite tendency, for the structural abnormalities characterizing the Albany strain as a whole to be most pronounced in those rats with mammary tumors.

Table III: Summary of the Statistical Analysis Made on the Quantitative Data from Anterior Hypophyses of Albany Rats with and without Mammary Tumors

Age, months	Number of rats	Type of rats	Percentage of cells: Eosinophils	Granular basophils	Non-granular basophils	Chromophobes
17 to 28	61	No mammary tumors	M.17.8 ± 0.4 SD.4.0 ± 0.3	M. 2.0 ± 0.1 SD.1.3 ± 0.08	M. 3.1 ± 0.1 SD.1.4 ± 0.09	M.76.9 ± 0.5 SD.5.2 ± 0.3
	51	Mammary tumors	M.15.7 ± 0.3 SD.3.4 ± 0.2	M. 2.2 ± 0.1 SD.1.2 ± 0.07	M. 2.3 ± 0.1 SD.1.2 ± 0.07	M.79.6 ± 0.4 SD.4.4 ± 0.2
Significance ratio * of difference between means			4.2	1.4	5.6	4.5

* Significance ratio was calculated by the formula

$$\frac{\text{Mean 1} - \text{Mean 2}}{\sqrt{(\text{P.E. Mean 1})^2 + (\text{P.E. Mean 2})^2}}$$

When the ratio equals 3 or over the difference is considered significant.

DISCUSSION

Comparative studies of the anterior hypophyses of the Albany strain, a high mammary tumor strain manifesting a variety of functional endocrine disturbances, and the Vanderbilt strain, which is normal in all respects, show definite structural differences between the anterior pituitary glands and the ovaries of the two strains. These manifest themselves early in life and continue to be present, often with increasing intensity, throughout the lives of the animals. Since growth and reproductive function are normal in the Vanderbilt rats and obviously abnormal in the Albany strain,

the structural differences between the two strains must be considered as evidence of structural deficiences in the Albany rats. The fact that such abnormal structural characteristics exist in the pituitary glands of the Albany rats seems to support the hypothesis that this strain is characterized by an inherent anterior lobe deficiency. Since functional disturbances, such as the partial failure of ovulation, the high incidence of fetal resorption, and the retardation of growth, all point to a decrease in anterior lobe function, it becomes important to determine, if possible, just how the structural deficiencies in the anterior lobe are related to the functional deficiencies manifested by the strain.

Unfortunately, on the basis of present knowledge, no satisfactory correlation between the structure of the anterior lobe and its functional capacities is possible. However, several observations do seem pertinent. It has been shown that the most obvious structural deficiency in the anterior pituitaries of the Albany rats is the decreased percentage of eosinophils, and that the most obvious functional deficiency is the failure of ovulation and the formation of corpora lutea. In previous studies Wolfe and his associates (15–17) showed that release of secretion from the eosinophil was associated with luteinization in the ovaries, a view subsequently stated by Severinghaus (11). Still later it was demonstrated that the luteinizing hormone of the anterior lobe also played a role in helping to bring about the actual rupture of the follicle (6).

These findings immediately suggest that the relative deficiency of the eosinophils in the anterior pituitaries of this strain are related to the failure of ovulation and luteinization. Although this hypothesis seems logical, and is we believe correct, it brings up the difficult question of the relation between the structure of a gland and its functional activity. Since the eosinophils present in the anterior lobe must be considered as a measure of the eosinophilic secretion stored in the gland, the deficiency of eosinophils in the anterior pituitaries of the Albany rats would seem to indicate a deficiency of hormone storage. However, the functional abnormalities encountered in this strain would indicate that there is a deficiency in the amount of hormone released into the blood stream. Does the fact that there is a deficiency of hormone storage by the eosinophils of the Albany rats indicate that there is also a deficiency in the amount of hormone elaborated and released into the blood stream? Since the eosinophils have long been considered the source of the growth-stimulating hormone of the anterior pituitary, the same question arises in connection with the retardation of growth in the Albany rats. Unfortunately the answer is not now apparent, and until it is it seems impossible to determine the exact significance of the low levels of eosinophils in the endocrine disturbances that are present in the rats of the Albany strain.

Regardless of the mechanism involved, two previous studies closely associate a deficiency of eosinophils in the anterior pituitary with retardation of growth and a certain degree of failure of the reproductive system. In several strains of highly inbred guinea pigs that were characterized by poor growth and inferior reproduction, decreased levels of eosinophils were found in the anterior pituitary (21). In a strain of dwarf mice in which there was almost a complete failure of growth after the second week and pronounced retardation of the development of the genital system, eosinophils were completely missing from the anterior pituitary, although the presence or absence of basophils was not determined (13).

The relationship between the structural and functional endocrine abnormalities and the high incidence of spontaneous mammary tumors in rats of the Albany strain is at present obscure. It is not known whether these abnormalities in some way actually contribute to the development of tumors, whether they and the tumors are the result of a common constitutional defect, or whether the two phenomena are entirely unrelated and their simultaneous appearance in this strain a coincidence. However, since the endocrine

TABLE IV: SUMMARY OF THE STATISTICAL ANALYSIS MADE ON THE QUANTITATIVE DATA FROM THE OVARIES OF ALBANY RATS WITH AND WITHOUT MAMMARY TUMORS

	Number of rats	Corpora lutea per ovary	Normal follicles per ovary	Atretic follicles per ovary	Rats with wheel cells, per cent
No mammary tumors	39	M. 8.6 ± 0.8 SD.7.8 ± 0.6	M. 4.0 ± 0.2 SD.1.9 ± 0.1	M. 5.8 ± 0.5 SD.4.3 ± 0.3	84.2
Mammary tumors	37	M. 6.4 ± 0.8 SD.7.4 ± 0.6	M. 3.0 ± 0.2 SD.2.3 ± 0.2	M. 5.9 ± 0.5 SD.4.5 ± 0.4	78.4
Significance ratio * of difference between means.....	—	2	2.7	0.1	—

* Significance ratio was calculated by the formula

$$\frac{\text{Mean 1} - \text{Mean 2}}{\sqrt{(\text{P.E. Mean 1})^2 + (\text{P.E. Mean 2})^2}}$$

When the ratio equals 3 or over the difference is considered significant.

disturbances that characterize the Albany strain as a whole are most pronounced in the animals with tumors, the relationship seems to be more than a coincidence.

The possibility that there is an actual relationship between endocrine abnormalities and mammary tumor development has been strengthened by a recent analysis of the distribution of tumors in rats of this strain (3). The analysis shows that tumors have appeared more frequently in sterile than in fertile rats. This finding may be of great significance since it suggests a specific relation which, if eventually proved, may be of importance in helping to determine the etiological factors concerned in the inception of these tumors. It is well known that the lobule-alveolar system of the rat mamma normally attains its maximum growth during pregnancy. As a result of the endocrine deficiencies, the frequency of pregnancy in rats of the Albany strain is much reduced. Because of the diminished incidence of pregnancy, it seems logical to assume that the degree of structural and functional development of the mammary glands does not reach the level found in more normal strains. One would expect, moreover, that such developmental deficiencies would be most pronounced in the sterile rats. If it can ultimately be established that fibroadenomas originate most readily in poorly developed mammary tissue, the relationship between the endocrine dysfunction and the appearance of mammary tumors would then be quite definite, since the poor mammary development is the direct effect of decreased ovarian activity in this strain.

SUMMARY

A comparative histological study was made of the anterior hypophyses and the ovaries of the Albany and Vanderbilt strains of rats. The Albany strain is characterized by certain endocrine abnormalities suggestive of an inherent hypofunction of the anterior pituitary and by a high incidence of spontaneous benign mammary tumors. The Vanderbilt rats do not show any evidence of endocrine abnormalities, and mammary tumors appear infrequently. In addition, the comparative effects of advancing age on these organs in the two strains were studied in rats ranging from 1 month to 28 months of age.

In all age groups it was found that the percentages of eosinophils in the anterior hypophyses of the Albany rats were significantly lower and those of the chromophobes significantly higher than in the Vanderbilt rats. In contrast, the relative number of the basophils was practically the same in the two strains.

In both strains advancing age was associated with definite structural changes in the anterior hypophyses and the ovaries. In the anterior lobe these alterations included a progressive decrease in the percentages of the eosinophils and an increase in those of the chromophobes; a decrease of mitotic activity; the appearance and increase in the numbers of vacuolated basophils; the appearance and increase of colloid degeneration of the anterior lobe cells and the deposition of intercellular colloid; the appearance and increase of colloid in the residual cleft; and the appearance of adenomatous changes. With the exception of the latter, all these structural alterations occurred earlier and were more intense in the anterior pituitary glands of the Albany rats.

In the ovaries advancing age was associated with a progressive decrease in the numbers of normal follicles, total follicles (normal plus atretic), and corpora lutea. There was a partial failure of ovulation and an increase of follicular atresia. An increase of interstitial tissue occurred and wheel cells appeared and became more numerous. All these changes took place much earlier and were more intense in the ovaries of the Albany rats. The histological findings in the anterior hypophyses and ovaries have been accepted as evidence that structural manifestations of advancing age appear prematurely in these organs in the Albany rats.

The nature of the endocrine abnormalities in the Albany strain is considered and their possible relationship to the appearance of the mammary fibroadenomas discussed.

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A Cancer Family Manifesting Multiple Occurrences of Bilateral Carcinoma of the Breast*

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In most studies pertaining to carcinogenesis in man there has been a tendency to disregard or minimize data obtained clinically. As Weller (10) and others have clearly shown, however, pertinent clinical data, do come from a number of sources,—"cancer families, family histories of cancer patients, multiple tumors in the same patient, familial concentration of certain neoplasms, and the tumors of monochorial twins." Many of these data are difficult to interpret because of their imperfect quality and the complexity of human genetics. From time to time we are apt to encounter "cancer families" where it seems that the incidence of neoplasia is significantly in excess of the normal expectation.

The reporting of the more significant or outstanding "cancer families" not only should be of immediate value, but should stimulate us and our successors to focus more critical attention on such families, resulting in careful periodic reports. Such a report has been made by Hauser and Weller (3) on a family originally reported by Warthin (8). Possibly in this way, after many generations, the present imperfect quality of information obtainable from the histories of cancer families may be improved and the information rendered more precise. Concomitant with this there will be an increased emphasis on obtaining accurate tissue and necropsy reports upon all members of any given family. The clinician can improve the quality of the data in this way. As the factual knowledge in various families becomes more complete, data from this source may be given to the geneticist for interpretation.

The family to be reported is one that manifests a number of interesting features and on which subsequent reports should be of value. In this family, whose record we have for four generations, there is an unusual incidence of bilateral carcinoma of the breast. The breasts have uniformly exhibited well developed, almost excessive, acinar and duct structures, as well as carcinomas of similar structural type. Even though a definite tendency to mammary cancer seems to be inherited on the maternal side, only those who are nursed by their mothers develop it. Thus, it is noted that cancer-free mothers have given birth occasionally to siblings who subsequently developed cancer. Histories of these cancer-free mothers show that even though they had not been nursed by their mothers, they possessed maternal ancestors afflicted with breast cancer. One noncancerous woman, the mother of three cancerous siblings, had not been breast-fed. A cousin on her maternal side, however, died of bilateral cancer of the breast. The similarity as regards the tissue changes in the involved and noninvolved portions of the respective breasts is striking in all the patients with breast cancer. Are these various interesting observations coincidental, or have they some specific significance?

Data from four generations are available (Fig. 1) in this study and are most complete on the maternal side.

Our attention was directed to this family while studying three sisters of the third generation (Fig. 2). Two had already developed bilateral cancer of the breast and three years ago, at the age of 50, the third sister presented herself with a carcinoma for which a radical mastectomy was performed. Fig. 2 shows that a daughter (fourth generation) of one of these women afflicted with bilateral carcinoma of the breast developed a mammary cancer at the age of 18 years. This was followed in one year by the removal of a low grade fibromyosarcoma from the chest wall. Two and a half years after removal of the left breast, a small innocent looking lump was discovered in the upper inner quadrant of the right breast. In view of the family history, a simple mastectomy was performed.

The family in its four generations is shown graphically in Fig. 1. Members of the first generation were born and raised in Germany. Because of this, information is limited to those members who emigrated to the United States and to their children. On the paternal side in the second generation the two members who did come to this country both died with carcinoma. Their brothers and sisters remained in Germany and are reputed to have died without the development of malignant growth, although their children are said to "have had many operations for tumors." On the maternal side the record is much more complete, and

* Read before Third International Cancer Congress, Atlantic City, September 15, 1939.

it is noted that in the first generation a niece of the transmitter emigrated to the United States, where she developed bilateral cancer of the breast and died of the disease at the age of 40 years. She was the first four generations, we feel that a compilation of percentage incidence would be meaningless. It is worth while to point out, however, that in the nine people afflicted with cancer, the primary site was the breast

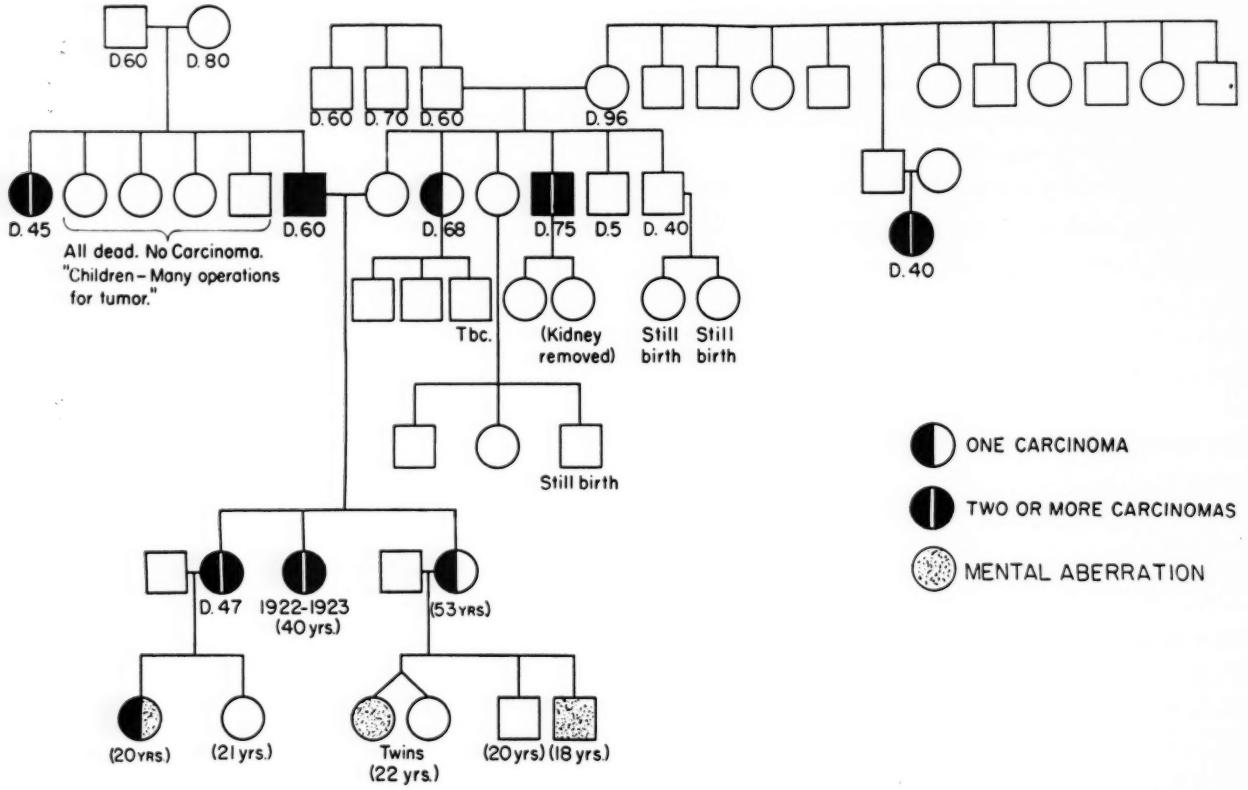

FIG. 1.—Family history for past four generations. Ages stated are either those at time of death or, of living members, at the time the paper was read in 1939.

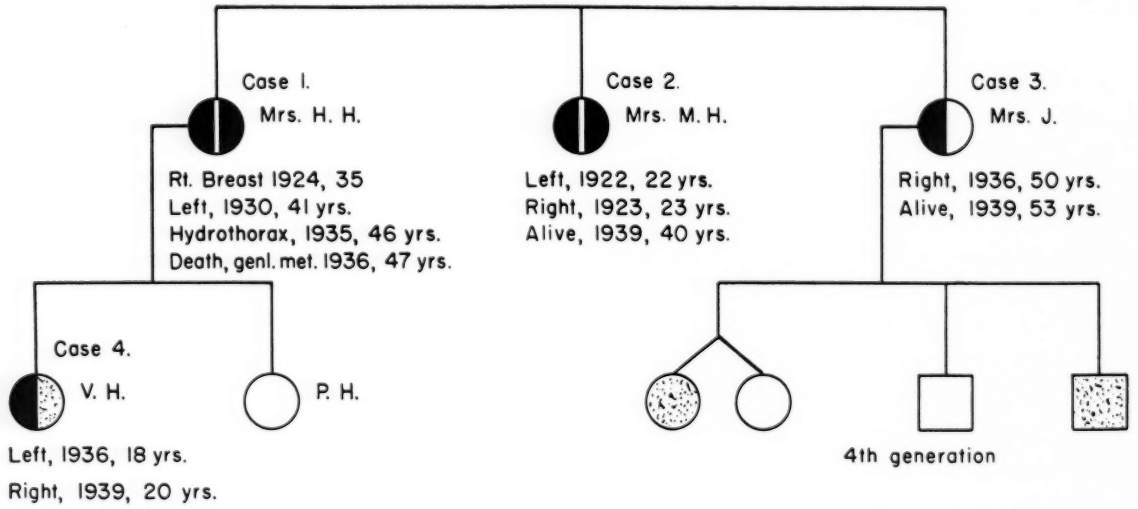

FIG. 2.—Sisters of the third generation with offspring noted.

cousin of the transmitter in the second generation whose three daughters and one granddaughter developed carcinoma of the breast.

Inasmuch as considerable gaps exist in our data, and in view of the fact that our study pertains to only in six, the colon in two, and the uterine cervix in one. One of the males with carcinoma of the colon also had a basal cell carcinoma of the face. Significantly, the predisposition to malignant growths has manifested itself by susceptibility on the part of the breast. Five

of the six instances of mammary cancer were on the maternal side.

The nursing history is of interest. So far as it goes it is compatible with the observations of Bittner (1) in mice pertaining to a "milk transmissible extrachromosomal influence" in the etiology of mammary tumors. A study of the maternal line of descent (Fig. 3) reveals that in the first and second generations the predisposition to mammary cancer was transferred by transmitters who were themselves free of breast cancer. As previously mentioned, the transmitter of the first generation had a niece who died of bilateral carcinoma of the breast. This same transmitter was unable to nurse any of her six children. She herself was one cancer of the face; the other, a female, a cancer of the cervix.

In passing, one might raise the query as to whether or not the patient with carcinoma of the cervix escaped mammary cancer because she had not been nursed by her mother. Hypothetically, one could postulate that in infancy her breasts had not been "sensitized" by the "breast-cancer-producing influence of Bittner" transmitted in the milk of her mother. It has been shown by a number of workers experimentally that estrogens are of etiologic importance for cancer development in both the breast and the uterus. Significant also is the fact that this one patient is the only one who developed cancer of the cervix.

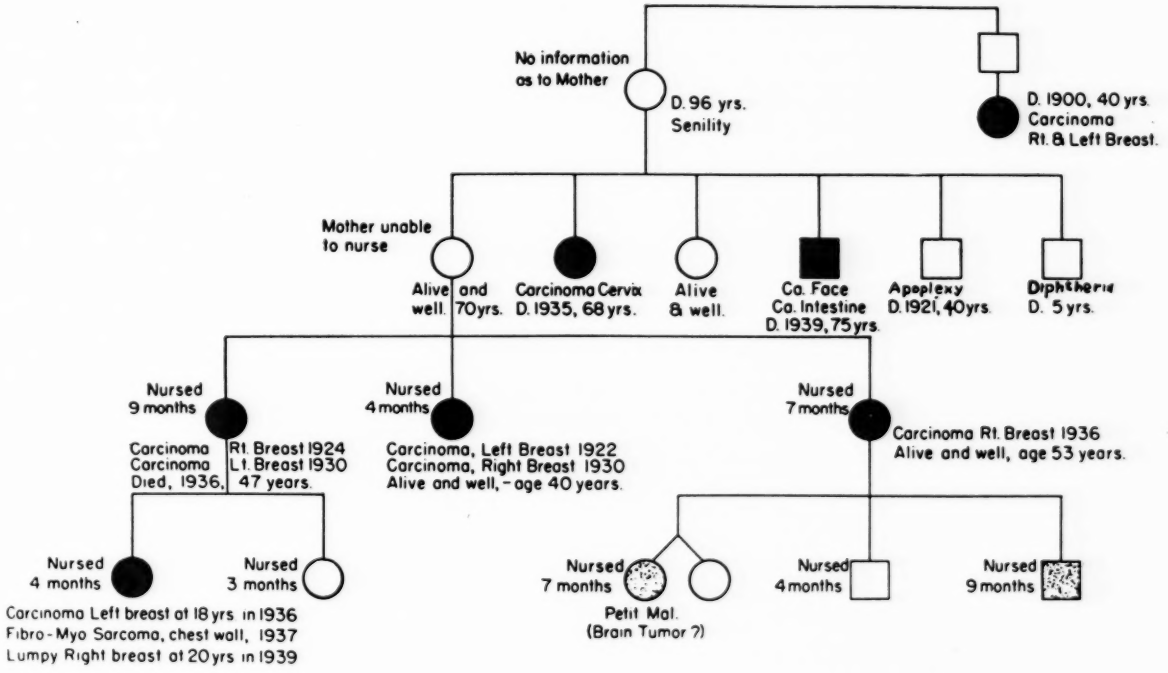

FIG. 3.—Maternal line of descent and nursing history for four past generations.

of a family of twelve children. We have no information as to whether or not her mother had been breast-fed. One of her daughters, who became the transmitter for the second generation, is alive and well at the age of 70 years and is free of cancer. This woman nursed all three of her daughters, and all three developed mammary carcinoma. Two of these three married, had children, nursed them, and already one at the age of 18 years has developed a breast cancer. The nursing history and its relationship to the development of mammary cancer is shown in Fig. 3.

In reiteration, one is impressed by the fact that even though the transmitter of the first generation had an afflicted maternal niece, the transmitter did not nurse any of her six children and none developed breast cancer. Two, however, developed cancer at other sites; one male, a cancer of the colon and a basal cell

In the third generation two of the three women, all with breast cancer, married and had children. One nursed her two daughters four and three months each respectively. She had difficulty in nursing her babies, and ultimately had to resort to supplementary feeding. As already mentioned the younger of these two daughters developed mammary cancer at the age of 18 years. There are five other members of the fourth generation (see Fig. 3), three of whom are females.[1] They are all approximately twenty years of age. All were nursed by their mothers. What will a report twenty or forty years hence reveal as regards their fate?

It is striking that in all the cases in which material was available for histological study similar tissue changes existed in the noninvolved as well as the in-

[1] Since this paper was read in 1939 one of the twin sisters in this generation has succumbed to glioma of the brain.

volved portions of the various breasts. Characteristically, there was a notable adenomatoid hyperplasia with irregular perilobular and periductal fibrosis, associated with carcinomas of the small duct variety. Possibly this is a manifestation of hyperestrinization. Photomicrographs of the tissue in case 3 are representative (Fig. 4). The case histories of the four afflicted women of the third and fourth generations, shown in Fig. 3, are herewith presented.[2]

Case 1.—Mrs. H. H., age 35 years. On Aug. 4, 1924, a radical mastectomy of the right breast was performed (surgical specimens BB-427 and BB-444). A poorly defined, hard mass about 1.5 cm. in diameter was situated in the lower inner quadrant. It was not connected with either the skin or the deep fascia. Present in the axilla were several moderately enlarged, soft lymph nodes. Aside from the tumor the breast appeared diffusely fibrous and contained but scant adipose tissue. There were many small ducts, which were dilated and filled with semifluid yellowish material. Histological sections revealed a carcinoma of the small duct variety, elsewhere considerable adenomatoid hyperplasia of the mammary tissue. No tumor was found in the axillary lymph nodes.

Nearly 6 years later, on May 2, 1930, the left breast was removed by radical mastectomy. The specimen (GG-66) contained a poorly defined, hard mass 2.0 cm. in width situated just beneath the nipple. Otherwise the breast showed a moderately diffuse induration with increase in fibrous tissue. Lymph nodes in the axilla were moderately enlarged and soft. Microscopically the lesions were essentially similar to those found in the right breast, which had been removed 6 years previously.

On Nov. 16, 1935, 5½ years later, a hydrothorax was tapped and its sediment revealed metastatic adenocarcinoma (JJ-409). Nine months after this episode tumor cells were found in ascitic fluid. The patient died in the latter part of 1936, having survived each tumor 6 years.

Case 2.—Mrs. H., age 22 years. On Jan. 17, 1923, a radical mastectomy of the left breast was performed (surgical specimen AA-69). The breast contained a poorly defined mass of very hard consistency that measured 1.0 cm. in width. Histologically the tumor consisted of small duct-like structures lined by polyhedral epithelial tumor cells. Elsewhere the acini and ducts were numerous and well developed.

One month later the right breast also was removed by radical mastectomy (AA-138). This breast also showed carcinoma, abundant fibrous tissue, and a pronounced development of ducts and acini.

On both sides the tumor was well localized and had not spread to the axilla. This patient is alive and well 16½ years following operation. She has had no children.

Case 3.—Mrs. J., age 50 years. This patient was the eldest sister in the third generation, and the last to develop carcinoma. On Dec. 10, 1936, radical mastectomy of the right breast was performed. Deep beneath the nipple was a poorly defined, hard tumor that measured 1.5 cm. in width. Microscopically it consisted of small duct-like structures supported upon a stroma of moderate thickness. The noninvolved breast tissue showed a definite glandular hyperplasia (Fig. 4). No tumor was found in the axillary lymph nodes. This patient, who is now 53 years of age, is alive and well.

Case 4.—Miss V. H., age 18 years (daughter of case 1). On Dec. 17, 1936, a radical mastectomy of the left breast was performed for a small, poorly defined mass 1.0 cm. in width (surgical specimen KK-659). Present throughout the breast was a diffuse increase in fibrous tissue. Microscopically the tumor was not unlike areas found in the three cases referred to above. A few ducts, however, were dilated and showed papillomatous piling up of the lining epithelium. Elsewhere the mammary tissue showed a noteworthy adenomatous hyperplasia, associated with considerable increase in inter-, intra-, and perilobular fibrous tissue. None of the axillary lymph nodes contained tumor. They were, however, enlarged and soft.

Two and a half years later the patient was about to be married and consulted her family physician for advice. Examination revealed a small nodule situated medially in the upper portion of the right breast, about 1 inch from the areola, and approximately 0.5 cm. in width. None of the classical signs of malignancy was present. The nodule had been present for some time. In view of the family history, and the uncertain nature of the "lump," mastectomy was performed Apr. 4, 1939 (surgical specimen 39S-657). The nodule consisted of a number of small ducts and irregular periductal fibrous tissue. Elsewhere there was apparent on gross examination a moderate increase in glandular and supporting fibrous tissue. Microscopic sections taken from various places showed a moderate, rather uniform hyperplasia of the glandular epithelium that in certain areas, however, had undergone subsequent atrophy. The outlines of periductal and perilobular collagenous connective tissue were indistinct. A section through the nodule showed a rather large collection of closely approximated dilated ducts, lined by small cuboidal to polygonal epithelial cells. The glands (acini) of these respective ducts showed definite atrophy. A few of the ducts contained homogeneous, deeply stain-

[2] The authors are indebted to the late Dr. Wm. Ophüls, the late Dr. Emmet Rixford, and Dr. Emile Holman for reference to their records in the collection of these data.

ing, slightly calcified plugs. All these changes were present also in the noninvolved portion of the left breast removed 2½ years previously.

DISCUSSION

The family history just presented shows a general susceptibility to cancer with a predisposition to the development of cancer in the breast, frequently bilateral, for when it developed it was in this organ in all but one woman in the second generation, in whom it was in the cervix.

The possibility has been considered that an "extrachromosomal breast-cancer-producing influence transmitted in mother's milk" might have been active in the various patients in which carcinoma of the breast had developed.

The family history shows no inbreeding, and in the main line of descent only one father had proven carcinoma (of the colon). Thus it would seem likely that a monohybrid recessive factor exists. One would assume from the data available a semidominant form of inheritance. This is compatible with the studies in human genetics made by Martynova (6). Such a semidominant factor should enable this family to be followed for many generations.

In none of our patients did so called chronic cystic mastitis, or cystic disease of the breast, occur. No blue dome cysts were present. All, however, showed a definite adenomatoid and small duct-like hyperplasia associated with a diffuse and irregular proliferation of fibrous tissue. It is suggested that this pronounced mammary gland development is a manifestation of hyperestrinization. In each of the breasts studied these changes were essentially of the same order and degree. The epithelial hyperplasia and fibrosis suggest an antecedent process of some duration. Changes in the right breast of case 4, which was removed prophylactically, are similar to those in the noninvolved portion of the left breast removed 2½ years previously. It is conceivable, therefore, that carcinoma would ultimately have developed in the right breast too.

The average age at which cancer appeared in the four instances just reported (see Fig. 3) is 32 years, which is much lower than that for the general population. The other two women with cancer of the breast succumbed at approximately 40 years of age but because their exact ages are not available, they are not included in the computation.

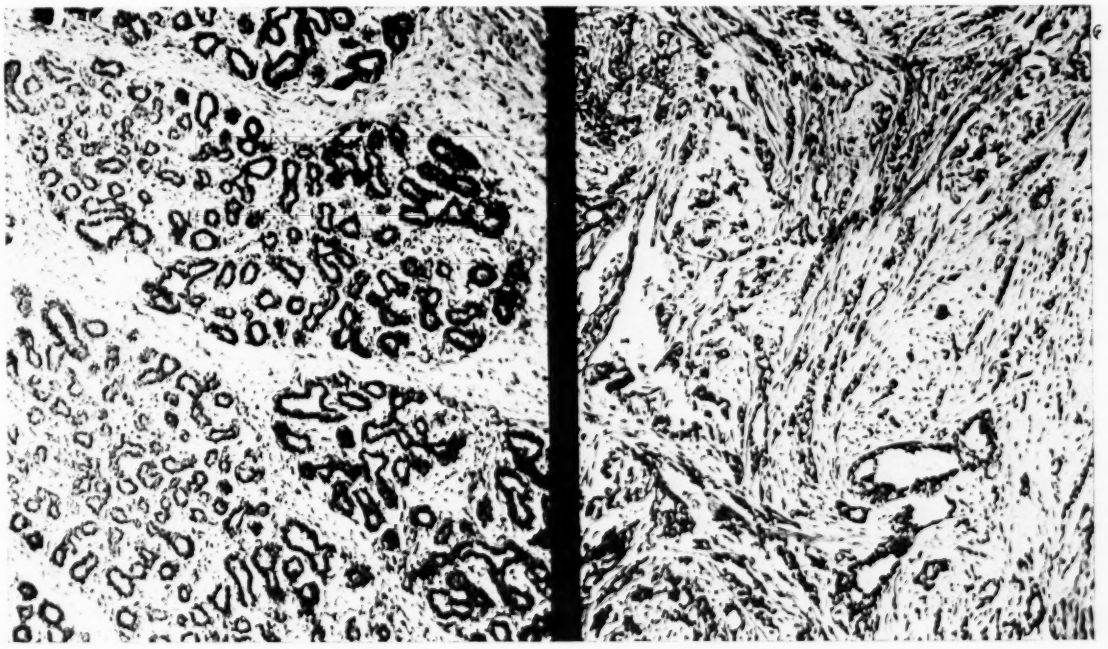

FIG. 4.—Pronounced glandular hyperplasia of noninvolved portion of breast. The print to the right shows a small duct carcinoma with a moderately fibrous stroma (case 3). Mag. × 100.

The data are so scant that one is not justified in stating whether the incidence of cancer in this particular family is increasing or decreasing. It would seem, however, that there is a trend in the former direction. The diagnoses of all tumors listed in the charts are well authenticated histologically except for the case of the paternal sister in the second generation. Slides from this case are not available for study.

It would seem from the study of this family that *stagnation* in the mammary gland is of little or no importance as a factor in the development of cancer. It is significant that, in spite of a predisposition to

cancer running through the maternal line of descent, in the first generation the mother was unable to nurse any of her six children, yet escaped cancer of the breast and lived to the age of 96 years. In the second generation the mother nursed her three girls and escaped cancer. It is to be noted, too, that she escaped both supposedly important carcinogenic factors—stagnation, and the extrachromosomal factor of Bittner. In the third and fourth generations mammary carcinoma occurred in childless women at the ages of 22 and 18 years respectively. In so far as we know there had existed no antecendent pregnancies with abortions in either of these two cases.

Most of the women in this family have slight brownish pigmentation of the skin and show an increased pilosity of the face. Whether or not this is indicative of hyperfunction of the adrenals is hard to say. There is no evidence of pituitary dysfunction.

It is suspected that a favorable "maternal nursing factor" in the presence of active hyperestrinization is especially important in the subsequent development of mammary cancer. Lacassagne (4), Loeb and his associates (5), and others have demonstrated the effect of hyperestrinization in the histogenesis of mammary cancer in mice. Examination of the mammary tissue in our cases suggests the intervention of hyperestrinization because of the abundant mammary glandular development.

The occurrence of mammary carcinoma in this family shows the same trends that were noted in the statistical studies of Waaler (7) in Norway, and Wassink (9) in Holland. Waaler demonstrated that the cancerous siblings of patients with cancer of the breast had an incidence of 44.7 per cent in contrast to 16.5 per cent of cancerous siblings from parents with cancer at other sites. To use Wassink's term, the "homotope organ" in this family is the breast.

A cancer family with five sisters afflicted with mammary carcinoma, in three of whom the disease was bilateral, was recently reported by Handley (2). In two of these patients there were changes that he described as "chronic mastitis," one proliferative in type.

When it is discovered that a patient is a member of a family such as the one just presented, or that cited by Handley, the question of early recognition of the disease as well as possible prophylaxis becomes pertinent. Which of the following, if any, should be suggested or recommended: A program of "wait and see," with periodic examinations? Surgical excision of the breasts? Administration of antiestrogenic hormones, or castration? Shall we be able to recognize in the future a syndrome indicative of hyperestrinization or other syndromes that might serve as danger signals? Should these women have babies? If so, should they nurse them, and if not, should they run the danger of breast carcinoma from stagnation? In our present ignorance it is obvious that no one feels competent to give direct answers to these various queries.

SUMMARY

1. A cancer family with data for four generations is presented in which bilateral carcinoma of the breast had occurred in a number of instances.

2. Attention was drawn to this interesting family during a study of the third generation. These were three sisters, all of whom had breast cancer.

3. One female sibling of the fourth generation developed a breast cancer at the age of 18 years.

4. The predisposition for cancer of the breast seems to be transmitted in the maternal line of descent.

5. Breast cancer occurred only in those women who had been nursed by their mothers.

6. Mammary glandular tissue in all cases examined histologically was hyperplastic and compatible with the changes induced by hyperestrinization.

7. The question of prophylactic advice and treatment is considered.

8. In view of the hyperplastic breast tissue and the rather singular nursing history the operation of a factor somewhat similar to that demonstrated by Bittner (1) in mice is suspected.

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Sebaceous Glands and Experimental Skin Carcinogenesis in Mice*

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The fluorescence microscopic study (5) of the absorption by mouse skin of methylcholanthrene in benzene has shown that a large portion of the carcinogen goes immediately to the sebaceous glands, where it is found dissolved in the lipid droplets within the gland cells. Another portion is absorbed by the keratinized layer of the epidermal epithelium, where it is dissolved in the free intracellular lipids of that layer. There is no evidence from these fluorescence studies that any of the carcinogen is absorbed directly by the living cells of the epidermis.

After a single application of methylcholanthrene the sebaceous glands degenerate rapidly and usually disappear completely by the fourth day. As the glands degenerate, sebum with its dissolved carcinogen is pushed into the hair follicles and, through them, onto the surface of the skin. Here it redissolves a part of the dry carcinogen remaining from the time of the painting. This layer of carcinogen-containing lipid bathes the surface of the skin until the keratin layer flakes off, usually at the sixth to eighth day. During this 6 to 8 day period localized areas of the epidermis and of the hair follicles become hyperplastic and show a tendency toward differentiation. The epithelial cells assume irregular sizes and shapes and often contain abnormal nuclei. Earlier work (1, 2, 3) had shown that these changes are often progressive, leading, in a considerable fraction of susceptible strain animals, to the development of carcinomas. In order to understand better the significance of the selective distribution and retention of methylcholanthrene, it was desirable to determine the reaction of skin to the carcinogen dissolved in a medium that resembles sebum.

Anhydrous wool fat (anhydrous lanolin) was chosen as a vehicle for this purpose since it presumably represents chiefly the secretion of the sebaceous glands of the sheep. We have no data on the composition of sebum from the mouse, but have assumed that it is similar to that of other species. 20-Methylcholanthrene was dissolved in the melted lanolin to a concentration of 0.3 per cent. This solution was melted (40–45° C. at the time of use) and applied by means of a brush to a large area of the back of each mouse.

* This investigation was aided by a grant from an anonymous donor.

Single Application

In the first experiment, 15 male and 15 female mice, laboratory bred from Swiss strain mice purchased of Tumblebrook Farm, were shaved over a large part of the back and to each was applied, after an interval of several days, a quantity of the carcinogen solution sufficient to contain approximately 0.3 mgm. of methylcholanthrene. This is the same amount of the carcinogen as was used in benzene solution by Cramer and Stowell (3) to produce malignant neoplasms in this strain of mice. Regrowth of hair occurred in these mice just as in untreated shaved mice. There were subsequently no skin changes to indicate that the mice had received treatment with a carcinogenic chemical. After 8 months the 26 survivors appeared to be perfectly healthy, normal animals.

Repeated Applications

The failure of methylcholanthrene in lanolin to induce any macroscopically visible change in the skin of the mice in the first experiment led us to consider the effects of continuous application of such a solution. Previously reported experiments (6) had shown that in Swiss mice thrice weekly applications of a 0.3 per cent solution of methylcholanthrene in benzene for 14 weeks caused the development of malignant tumors in 33 per cent of the mice by the end of the period of painting, in 50 per cent at the end of the 17th week, and in all the surviving animals at the end of the 26th week.

In the present experiment 50 mice of the Swiss strain were used. Since mice were not available from the source previously used (Tumblebrook Farm) these animals were purchased from the Albino Farms, Red Bank, New Jersey. Experiments with a benzene solution carried out at the same time indicate that the Albino Farm Swiss strain mice readily developed skin cancer. The carcinogen in lanolin was applied 3 times weekly for 14 weeks to the unepilated skin with an

average dose per application calculated to be approximately 0.2 mgm. This is from 2 to 4 times the dose that was given when thrice weekly paintings with benzene solutions were employed. Macroscopic examinations in ultraviolet light show that such a lanolin solution spreads uniformly over a large area of the back of the mouse, remaining semifluid from the heat of the animal's body. Fluorescence microscopic studies of skin painted with the methylcholanthrene-lanolin solution show that the absorption of the carcinogen in this vehicle is similar to the absorption of a benzene solution. Since the solvent, lanolin, is not volatile, there is no crust of dry, crystalline methylcholanthrene left on the surface of the skin and the only fluorescence observed is the blue-violet characteristic of the dissolved carcinogen. This appears in the sebaceous glands and in the keratin layer. After a single painting this fluorescence disappears in 2 to 3 days, which is much quicker than with a benzene solution. Even so it may be assumed that with the 3 paintings weekly the skin was exposed continuously to the carcinogen in lanolin for the entire 14 week period.

These results with the lanolin solution of methylcholanthrene are in striking contrast to those obtained with benzene solutions. Epilation, which is an early and regular occurrence with the latter method of application, does not occur. At the end of the 14 week period of painting, 47 mice survived. No tumors had appeared. A week after the painting was stopped a few mice were sacrificed and their skins were examined microscopically (Fig. 1). No hyperplasia was observed, although a slight increase in the amount of keratin was noted. Sebaceous glands were still present but much reduced in size and less numerous than in normal mice. Some increase was noted in the amount of fat within and beneath the dermis. None of the surviving mice had developed a malignant tumor at the end of 26 weeks. One mouse that died in the 23rd

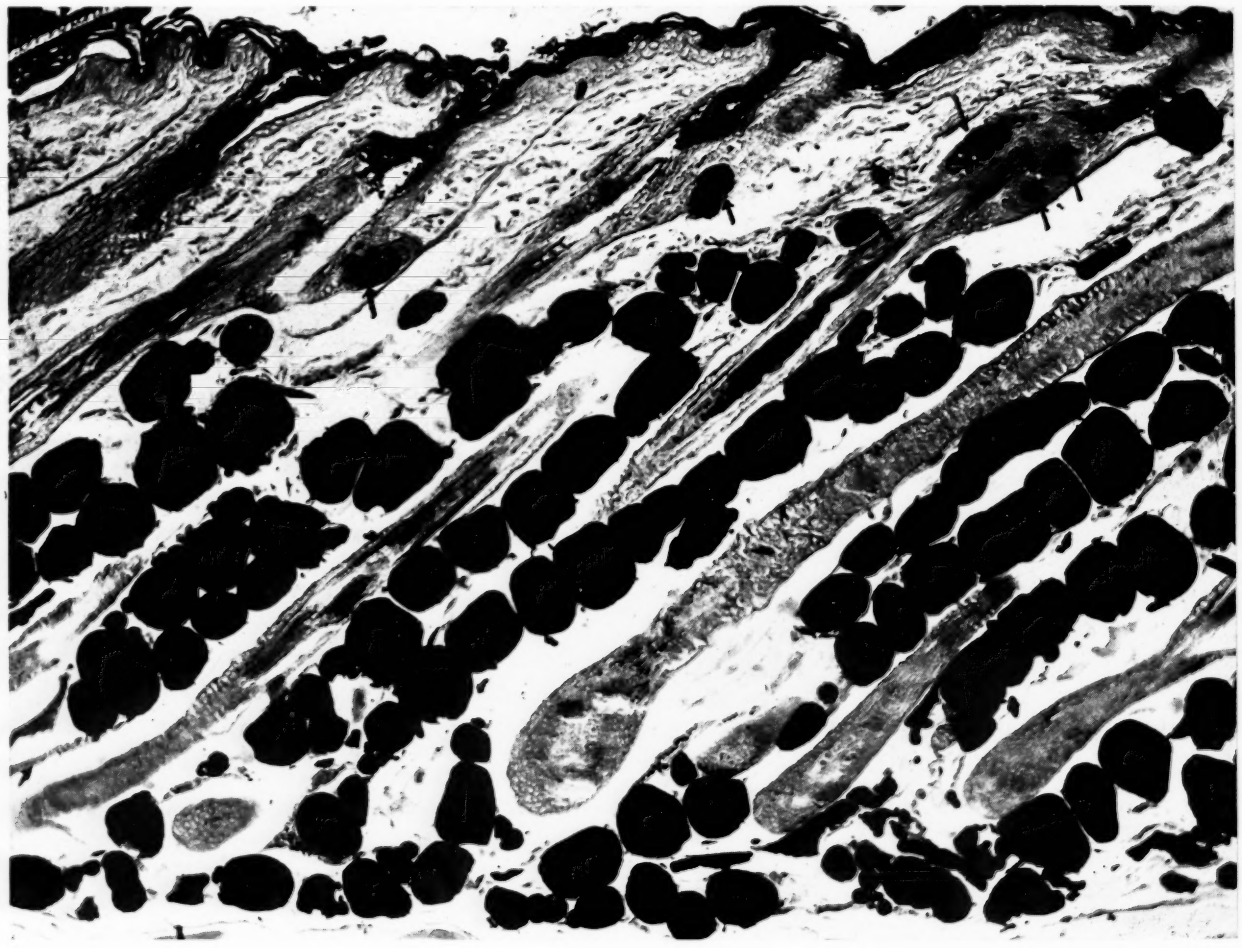

FIG. 1.—Axial section through the skin of a mouse 3 days after the 42nd painting with methylcholanthrene in anhydrous lanolin. The epidermis and hair follicles appear to be essentially normal. The layer of blackened fat cells is somewhat thickened and extends well up into the dermis. Sebaceous glands, indicated by arrows, are reduced in number and size and are irregularly distributed. The field was selected to show as many glands as possible. Unstained Schridde preparation. Mag. × 140.

week had a small, warty growth. Microscopic examination showed it to be a keratinizing papillomatous wart of precancerous character. Two other mice were found to have each one minute papilloma at the end of the 26th week. At this time, 12 weeks after the last application, a few more mice were killed for microscopic examination of the skin (Fig. 2). There was no hyperplasia of the epidermal epithelium. Sebaceous glands, having undergone some degree of restoration, were, if anything, larger than normal. There were no changes to indicate that the skin had been exposed to a potent carcinogen. With the exception of the 3 mice referred to above, the lanolin had completely protected the skin against the carcinogenic and other effects of methylcholanthrene. Throughout the experiment the animals were in good condition, the mortality was low, and many mice were heavier and more obese than mice treated with a benzene solution of methylcholanthrene.

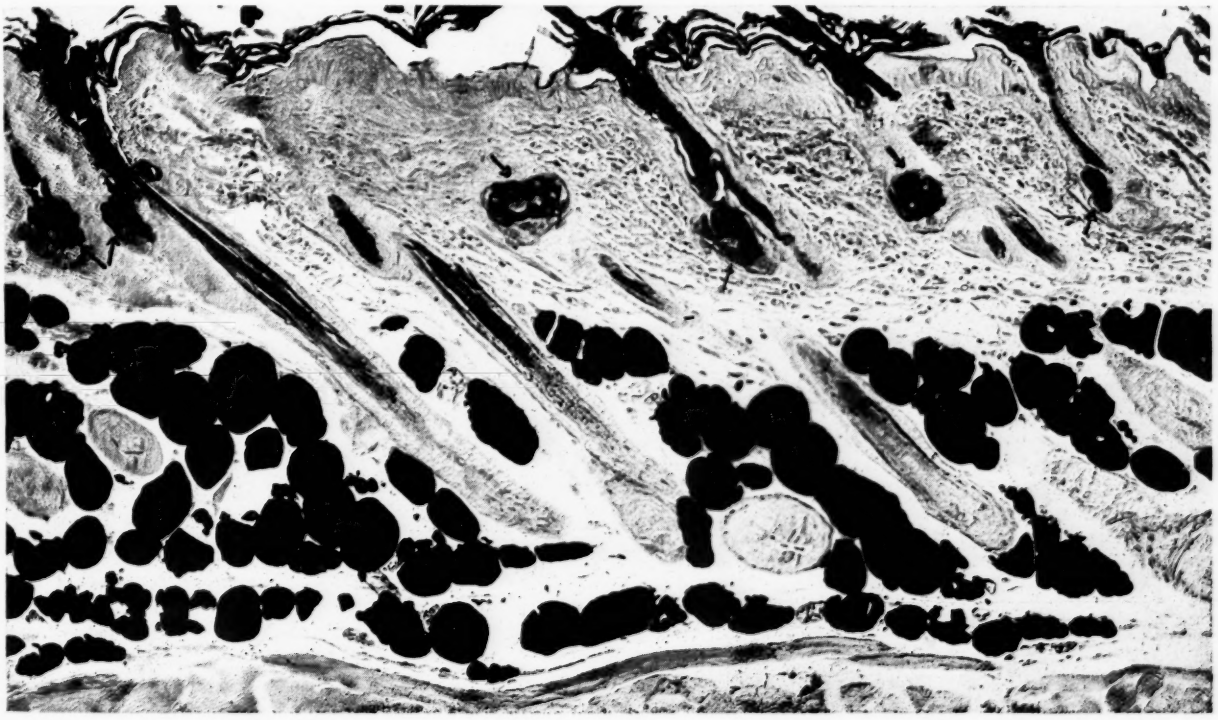

FIG. 2. Axial section of mouse skin 12 weeks after the last painting with lanolin solution of methylcholanthrene. The amount of subcutaneous fat is now approximately that of normal skin. The sebaceous glands, indicated by arrows, are restored. They are somewhat less frequent but are larger than the sebaceous glands of normal skin. There are no changes in the epidermis or hair follicles to show that the skin had been treated for 14 weeks with methylcholanthrene. Schridde preparation, unstained. Mag. × 140.

The experiment has been repeated with 60 more mice and at the time of writing—18 weeks after the first application of the lanolin solution of methylcholanthrene—the results are again completely negative.

Fluorescence Spectra

The possibility of a chemical combination between the carcinogen and anhydrous lanolin has been considered. Fluorescence spectra were made of methylcholanthrene dissolved in benzene and in anhydrous lanolin. These were compared only with the naked eye, a method believed by Hieger (4) to be more satisfactory than the use of a microphotometer. The position and relative intensity of the three characteristic bands in the blue-violet end of the spectrum appeared to be identical in spectra of the two solutions. Though this is but a crude check, it appears unlikely that a chemical alteration of the carcinogen has resulted from its solution in lanolin.

DISCUSSION

It is clear that, under the conditions of our experiments, solution of the carcinogen in anhydrous lanolin deprived the hydrocarbon almost completely of its carcinogenic property. Even after 42 applications of such a solution the sebaceous glands persist, though they are smaller and less numerous than in normal skin; the skin is not epilated; and there is no hyperplasia of the epidermis or of the hair follicles. This finding is in itself of interest, but it has, in addition, a special bearing on the fact that the carcinogen, when first applied in benzene solution to mouse skin, is selectively absorbed by the sebaceous glands and rapidly destroys them. Subsequent paintings with methylcholanthrene act on a skin area devoid of sebaceous glands.

Thus we find that methylcholanthrene in benzene, which is effective as a carcinogenic agent, first destroys the sebaceous glands, and that the same substance, in a vehicle resembling sebum, neither destroys sebaceous glands nor induces other changes leading to the development of cancer. Unless the sebum of mice differs essentially from that of sheep it would follow that the sebaceous glands provide a protective mechanism against chemical carcinogens.

Previous observations on the effect of a combination of lanolin with chemical carcinogens on experimental carcinogenesis have been made by C. C. Twort and J. M. Twort (7), but their results were contradictory. In 1930, when working with relatively weak carcinogenic agents such as shale oils and tars, they found that "the addition of animal and vegetable oil to the cancer producing material diminishes the capacity [to form cancer] far more than the dilution would lead one to suspect." Of the various oils used by the Tworts, lanolin had the most pronounced effect in diminishing carcinogenic potency. Later, in 1939, they (8) confirmed this statement with the qualification that it holds true only for dilute solutions or small quantities of carcinogenic gas tars. If, however, relatively large quantities or strong solutions of gas tars are applied, addition of lanolin to the tar will result in a greater yield of tumors than that observed among the controls treated with tar only. In experiments with dibenzanthacene the addition of "a small quantity of lanolin" to the chloroform solution increased "somewhat" the potency of the carcinogen. The technic of our experiments with methylcholanthrene differs from that used by Twort and Twort in their later experiments. They dissolved the carcinogen in chloroform and added a small quantity of lanolin to this solution, while in our experiments the carcinogen was dissolved in melted lanolin and no other solvent was used.

SUMMARY AND CONCLUSION

The carcinogenic activity of methylcholanthrene is almost completely suppressed when it is dissolved in melted anhydrous lanolin and the melted solution is applied to the skin of mice 3 times weekly for 14 weeks.

Lanolin respresents the sebum of sheep. Unless this sebum differs essentially from that of mice, our results suggest that the sebaceous glands act as a protective mechanism against this chemical carcinogen.

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Attempts to Induce Stomach Tumors

I. The Effect of Cholesterol Heated to 300° C.

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The rarity of gastric tumors in animals in contrast to the frequency of human gastric carcinoma has led to many and repeated attempts to induce stomach tumors in experimental animals. An exhaustive chronological catalogue of these attempts has recently been published by Sugiura (42). In the case of rodents, the stomach consists of two parts: (a) the forestomach, lined with squamous epithelium and separated sharply by a limiting ridge from (b) the glandular stomach; agencies affecting (a) may not affect (b). Forestomach lesions have been described as a result of at least thirteen distinct agencies, as set out in Table I.

Glandular stomach lesions have been much less frequently encountered and, until recently, no well authenticated case of adenocarcinoma had been recorded. Stewart and Lorenz (40), however, have now described the production of gastric adenocarcinoma in C3H mice, which have not been known to develop this lesion spontaneously, by the injection of 20-methylcholanthrene as a suspension in horse serum into the wall of the pyloric region. Although no metastases were observed in the 4 mice so far reported upon, penetration through the muscle wall into the peritoneum occurred and a transplant was found to grow and maintain the characteristics of the original tumor. Roffo (32–34) said that he had induced adenocarcinoma as well as sarcoma in rats fed fats or cholesterol heated to 300° C. His copious illustrations of the stomach lesions show advanced stages of ulceration but, as Klein and Palmer (22) point out, no evidence is offered of metastasis or penetration of the serosa; moreover, there is no record of any "adenocarcinoma" having been transplanted. Howes and Vivier (17) found ulcers widespread in the pars glandularis in areas of hyperplastic epithelium; the ulcers were craters with deep sides. Essentially similar lesions were obtained by Brunschwig and Rasmussen (5) in rats fed high carbohydrate or high fat diets. Howes and Vivier fed their rats with white flour and a salt mixture, that is, a high carbohydrate–low protein diet, but no lesions were found at 61 days in adult rats unless this diet were restricted in quantity also. Brunschwig and Rasmussen record experiments up to 160 days only. Since Roffo sets a minimum period of 16 months for the production of lesions, it is possible that these other workers would have obtained similar changes if they had continued their experiments long enough. Human stomach tumors are common only from later middle life onwards, and any investigations into lesions of the animal stomach must be designed to follow the natural life span as far as possible.

There is no doubt, however, that Roffo obtained severe lesions of the glandular stomach, and, to a lesser extent, of the forestomach, in rats, and further that he obtained genuine sarcomas by feeding fats heated for half an hour to 350° C. The maximum temperature to which any oven may be set is usually about 280° C., so that Roffo's temperature is definitely above that reached in ovens. Nevertheless, the same change or changes might be produced by repeated heating, especially in an open frying pan or grill, where the surface of the food may indeed attain a temperature of 350° C. or more. Roffo's results thus have an obvious importance from the human standpoint and a reinvestigation of his assertions was therefore undertaken in this department. The results of feeding repeatedly heated cooking fats and oils have been recorded by Beck and Peacock (1), who found no lesion of the glandular stomach in Norwegian hooded rats fed up to 436 days, save a hemorrhage in the last rat to die. No malignant changes were observed in the forestomach, but papillomatosis was a frequent feature. Controls never showed papillomatosis under 2 years and remained healthy, apart from a tendency to lung abscesses. The rats receiving heated fats or oils had the clinical signs of avitaminosis A, and this diagnosis was confirmed by estimations of the amount of vitamin stored in the liver; the vitamin reserve was depleted in proportion to the extent to which the ingested fat had been heated. Beck and Peacock therefore concluded that the heated fats contained some substance that interfered with vitamin A metabolism and so induced a deficiency of this vitamin. As a result of this deficiency, conditions

* Working under a full time grant from The British Empire Cancer Campaign.

TABLE I

Agency	Author(s)	Findings
1. Mechanical trauma	Bullock and Rohdenburg (7)	Polypoid growths *
2. Vitamin A deficient diet	Fibiger (11)	Coupled with *Spiroptera* infection
	Passey, Leese, and Knox (31)	Aggravated by *Spiroptera;* papillomas; occasional downgrowth
	Pappenheimer and Larimore (30)	Papillomas; same type of lesion as those of Buschke and Langer and of Fibiger
	Fujimaki (14)	Papillomas; no invasion †
	Fridericia *et al.* (13)	Papillomas; one "invasive"
3. Heated fats (induced vitamin A deficiency)	Beck and Peacock (1)	Papillomas; frequently ulcerated
4. Low protein diet	Howes and Vivier (17)	Not prevented by sources of vitamin A. Ulcers in hyperplastic epithelium; downgrowth; mitotic figures numerous
	Hoelzel and Da Costa (16)	Ulcers and papillomas
	Matzner, Windwer, and Sobel (24)	Ulcers (see also 8)
5. Vitamin B_2 deficient diet	Findlay (12)	Papilliferous hyperplasia; mitotic figures numerous
6. Choline deficiency	Sharpless (38)	Papillomas
7. Unbalanced ratios of fat, protein, and carbohydrate	Brunschwig and Rasmussen (5)	Ulceropapillomas. Mitotic figures frequent. Epithelial downgrowth not unrestrained
8. Intermittent starvation	Hoelzel and Da Costa (16)	More papillomas than by agency 4
	Matzner, Windwer, and Sobel (24)	Largely prevented by adequate protein
9. Starvation plus histamine	Büchner *et al.* (6)	Ulcers; deep downgrowths of epithelium
10. Tar	Buschke and Langer (8)	*Per rectum* ‡
	Tani (43)	*Per os;* papillomatosis
	Bonne (4)	In mice; *per os,* papillomatosis
	Twort and Twort (45)	Painting, mice; papillomatosis
11. Azo dyes	Bullock and Rohdenburg (7)	Localized papillomas; rats
	Otsuka (29)	Fed to mice; papillomatosis §
	Sasaki (36)	Fed to rats; papillomas
	Kinosita (20)	Fed to rats; many papillomas
	Kinosita and Harada (21)	Fed to rats; many papillomas
12. Cholesterol derivatives	Roffo (32–35)	Fed heat products
	Waterman (49)	Fed oleate to tarred mice; papillomas
13. Carcinogenic hydrocarbons fed to mice		
(a) Benzpyrene	Oberling *et al.* (28)	Hyperplasia
	Waterman (49)	Squamous carcinoma ‖
(b) Methylcholanthrene	Van Prohaska *et al.* (46)	Papillomas
	Lorenz and Stewart (23)	Squamous carcinoma

Experimental animals were rats, except where otherwise stated.

* Dietetic deficiencies not excluded.

† Low protein diet.

‡ Resemble lesions of Fibiger and of Pappenheimer and Larimore.

§ Otsuka has been erroneously reported by Klein and Palmer (22) to have used diaminoazobenzene, and by Sugiura (42) to have used aminoazobenzene. He actually fed a very different substance, diazoaminobenzene, as can be seen from the title of his paper (29), which was accurately quoted by Sugiura (42).

‖ Queried by Klein and Palmer (22).

were provided for the development of papillomatosis of the forestomach such as has been obtained in other cases of avitaminosis A (see Table I). It is probable, however, that the lack of vitamin A is only indirectly responsible. Thus Howes and Vivier (17) suggested that avitaminosis A would lead to loss of appetite and so to various other dietetic deficiencies, which might be the proximal cause or causes. This would explain the apparently anomalous results obtained by Wolbach and Howe (52), who found no stomach lesions in rats deprived of vitamin A; these workers prevented other deficiencies by forced feeding when appetites failed.

Beck and Peacock were unable to obtain any glandular lesions and some unrecognized factors in Roffo's experiments must have been responsible for the lesions he reported. The feeding of overheated oils and fats was found to produce only a shortage of vitamin A in the rat. Roffo suggested that oxidation of cholesterol was the cause of the carcinogenic properties acquired, according to him, by fats and oils on heating to 350° C.

It was therefore decided to investigate this statement and to ascertain what substance, if any, was produced that could cause gastric lesions in the rat. Wistar albino rats were obtained for the purpose from Glaxo Ltd., and the cholesterol used was also from this source. The stock diet in use in this department at that time was oats and water ad libitum, supplemented by kitchen scraps, including greenstuff. No stock or control rat had been seen to develop gastric lesions of any sort on this diet, but it was felt that a well balanced diet would be advisable to prevent any uncertainty as to the cause of lesions that subsequently might be found. A preliminary experiment to compare the value of this stock diet with that of the balanced diet worked out by the Rowett Institute (44) was definitely in favor of the latter, which was therefore adopted as the basal diet.

EXPERIMENTAL

Selection of Basal Diet

Thirty-six Wistar albino rats were placed in 9 cages with a random distribution of sex and size. The animals were about 3 months old when the experiment began, and weighed from 123 to 184 gm. Rats 1 to 20, in cages 1 to 5, were fed the stock diet of oats and water ad libitum, supplemented by kitchen scraps. Rats 21 to 36, cages 6 to 9, were fed rat-cake, supplied by the North Eastern Agricultural Co-operative Society, Ltd., Aberdeen, of which the daily consumption per rat was about 15 gm.; water was supplied ad libitum. At the end of 11 weeks it was clear that the second group, *i.e.* those on rat-cake, were gaining weight more rapidly than the first group, as illustrated in Table II.

It will be seen that the average weight gain of the least satisfactory cage, 7, of those fed rat-cake was greater than that of the best cage, 5, on the less controllable diet. Therefore all rats were maintained on rat-cake for the rest of their lives.

It was not found possible to prevent the development of lung abscesses, which tended to appear after 200 days, even by feeding fresh milk (about 30 ml. among 4 rats) and greenstuff once a week. The development of these abscesses was accompanied by a sharp fall in weight and usually death in a few weeks at most.

Table II

Cage Nos.	Average gain in weight per cage, gm.	Average gain in weight per group, gm.
1	5.0	9.35
2	5.5	
3	11.25	
4	12.25	
5	12.75	
6	19.0	17.8
7	14.25	
8	19.5	
9	18.5	

Preparation of Heated Cholesterol

A convenient quantity of cholesterol was melted in a beaker and the temperature raised to 270° C. by heating on an electric hot plate; the temperature was maintained at 270–300° C. for half an hour, during which time the color of the molten mass darkened, gases were evolved, and a small amount of white sublimate collected round the rim of the beaker. After half an hour, the temperature was allowed to fall to 150° C. or lower, and the molten mass was then poured with stirring into about 7 volumes of ethanol. When the ethanol solution had been cooled, the bulk of the unchanged cholesterol crystallized out and could be collected by filtration with suction and subsequently reheated. In this way almost all the cholesterol could be converted to heat products without subjecting the latter to more than half an hour's heating. One of the products of heating under these conditions was dicholesteryl ether, formed by the elimination of a molecule of water between two molecules of cholesterol; this was not soluble in hot ethanol and the alcoholic solution therefore had to be filtered before chilling. This inconvenience was overcome later on by substituting petrol ether (b.p. 60–80° C.) for ethanol; cholesterol is easily soluble in boiling petrol ether but soluble in cold petrol ether to the extent of only 0.6 per cent. Dicholesteryl ether has a solubility in cold petrol ether of about 2 per cent, and no difficulty was experienced in retaining it in solution, together with all the other nonvolatile products formed by heating cholesterol.

The combined petrol ether solutions from successive heatings were evaporated down and solvent removed as far as possible in a vacuum desiccator. The brown mass thus obtained was weighed and dissolved in chloroform to give a 20 per cent solution.

Feeding of Heated Cholesterol

One-tenth milliliter doses of 20 per cent solution were run on to pieces of rat-cake weighing about 3 gm., and the solvent was given ample time to evaporate. One such piece of rat-cake (20 mgm. of heat products) was fed each week day to each rat under experimentation.

Control rats received each week day a piece of rat-cake impregnated with 20 mgm. of unheated cholesterol.

The 9 cages of rats were divided into 3 groups, rats 13 to 20 and 33 to 36 (cages 4, 5, and 9) becoming controls. All the rest received heated cholesterol.

In order to ensure an adequate supply of vitamin A, a supplement of carrot, approximately 2 gm., was fed 3 times a week to all control rats (cages 4, 5, and 9) and a similar supplement to half the rats receiving heated cholesterol (cages 1 to 3), while no carrot was fed to the remainder (cages 6 to 8).

The diet was completed by feeding greenstuff and milk (about 30 ml. among 4 rats) once weekly, and rat-cake daily in such quantity that the rats were hungry next morning and would consume the impregnated piece at once.

RESULTS

No form of malignant growth was observed in any part of any rat. The majority of forestomachs were quite normal. In 3 of 12 controls the forestomach was the seat of minor changes. Of the 12 rats receiving heated cholesterol and no carrot, 1 showed minor changes; of 12 receiving heated cholesterol plus carrot, one exhibited early papillomatosis after 490 days, but 10 others dying at 527 to 730 days had only minor changes if any. Hence no significant effect was produced in the forestomach, either in the presence or absence of extra vitamin A. Slight hyperkeratosis and/or hyperplasia seems to be common after about 18 months of age.

Of the 33 stomachs that were available for postmortem examination, 24 showed changes in the glandular zone varying from areas of brown pigmentation to areas of hemorrhagic erosion. Of the 9 normal stomachs, 4 were from rats dying without any form of lung lesion. On the other hand, 27 rats died with lung abscesses and only 5 of these had normal stomachs; the remainder exhibited pigmentation and/or erosion, according to age.

Out of 25 stomachs from rats dying with lung abscesses, 20 were known to have had various stages of the process culminating in hemorrhagic erosion. One other rat, No. 11, dying at 669 days, had slight erosions but no lung lesions; however, this rat had advanced inflammatory destruction of the cecum. Three rats showed changes in the glandular zone, without abscesses in the lung or lesions in parts of the alimentary tract other than the stomach.

DISCUSSION

The chief significance of the results obtained from this series of experiments seems to be that Roffo's theory of the origin of cancer in the glandular stomach is not supported by the facts. It certainly appears that feeding products obtained by heating cholesterol to 300° C. for half an hour at a level of 20 mgm. per day does not produce any lesion in the glandular stomach, even after 2 years, which is beyond Roffo's minimum time limit. The existence of hemorrhagic erosions, and of stages leading thereto, does not seem to be related to the diet so much as to the actual food intake. Nearly all the rats with lung abscesses showed a sharp fall in weight during the fortnight or so preceding their death, and sat hunched up in the corner of the cage, making no attempt to seek food during the last day or two. These animals were probably more or less starving, and the same conclusion can probably be drawn about rat 11, which had so highly inflamed a cecum. In view of the work of Schioedt and Schültzer (37) and of Brunschwig and Rasmussen (5), it is not surprising that these rats should have developed such changes in the gastric mucosa. Findlay's (12) vitamin B_2 deficient rats often showed superficial ulceration of the mucosa that was probably related to the severe state of inanition in which they died rather than to the lack of vitamin B_2 *per se*. In any event, the hemorrhagic erosions were found in all 3 groups of animals, including 5 of 10 controls examined; they can hardly have been directly due to vitamin A deficiency.

Roffo's data regarding the basal diet of his rats are very scanty; his "stock diet" appears to be bread, of a type not specified, and milk. Hence it is extremely probable that his basal diet was deficient in most vitamins, in fat, protein, and possibly mineral essentials. The importance of a really balanced diet is emphasized by the preliminary dietetic experiment described in this paper. Deliberately unbalanced diets may be needed before a variety of potentially pathogenic agents can cause significant lesions of the stomach.

CHEMICAL ASPECTS

Roffo has frequently expressed his opinion that cholesterol irradiated with the ultraviolet beam or

heated in the presence of air undergoes an oxidative process whereby polycyclic hydrocarbons arise. At the same time, he states that the side chain of the cholesterol molecule is broken off to yield a substance, $C_{21}H_{36}O_3$, which is not a hydrocarbon. The evidence for this formula for his so called oxycholesterol is quite inadequate and, as Bergmann (2) has pointed out, would just as easily fit the formula $C_{27}H_{46}O_4$. A keto acid of this formula was obtained by Bergmann himself by ultraviolet irradiation of cholesterol. Moreover, Bergmann says, "One must assume that . . . the formation of a new ring is indispensable for obtaining a carcinogen." Hence whichever formula is correct for Roffo's derivative, the compound can scarcely be a carcinogen of a known hydrocarbon type since, in the one case, the side chain is eliminated and, in the other, one of the original rings has been opened.

Nevertheless, cholesterol heated even to 270° C. acquires a decided blue-violet fluorescence in the ultraviolet beam, reminiscent of that shown by carcinogenic hydrocarbons; a fluorescence spectrum, however, showed only general absorption. It was found that this blue fluorescent material was present in much greater quantity in the product obtained by heating cholesterol to 440° C. for half an hour. After repeated purification by passage down a tower of B.D.H. alumina "for adsorption purposes" in *n*-hexane, the substance in *n*-hexane showed broad fluorescence bands at 3,950, 4,200, and 4,400 Å. Roffo's assertion that the fluorescent product resembles 3,4-benzpyrene is not substantiated by this result, as 3,4-benzpyrene in *n*-hexane shows only 2 bands at 4,050 and 4,300 Å. However, the 3 bands obtained were so close to those of 20-methylcholanthrene in ethanol (4,000, 4,250, and 4,450 Å) that further investigation was essential. Mayneord and Roe (27) heated cholesterol in air to 195° C. and divided the product into ether- and alcohol-soluble fractions. The former had an absorption band at 2,550 Å, while the latter had a band at 2,350 Å and a "step-out" at 2,660 Å, which Mayneord and Roe assumed to be due to the presence of the ether-soluble material. The crude fluorescent substance in the present experiment was found to possess a sharp absorption band having its maximum at 2,550 Å. Further purification by chromatography yielded a solution from which a more detailed absorption spectrum could be obtained. This spectrum showed a series of bands with maxima at 2,330, 2,440, 2,560, and 2,615 Å. No trace of a band at any longer wave length could be seen, with either stronger or weaker solutions. Hence it would appear certain that this blue fluorescent substance is not one of the known carcinogenic hydrocarbons, all of which have bands in the region of 3,000 Å (26, 18).

That carcinogenic properties can be acquired by cholesterol on pyrolysis is shown by the work of Kennaway and Sampson (19), who obtained a large percentage of malignant tumors by painting mice with tar produced by vaporizing cholesterol in a stream of hydrogen at 800° C., and cooling the gases after passage over pumice at 800° C. It seems highly improbable that the cholesterol could have been converted to a substance such as methylcholanthrene, a process involving very thorough dehydrogenation, in a system saturated with hydrogen. It may be, therefore, that the pyrolysis of cholesterol yields a different type of carcinogen that may or may not be identical with the fluorescent substance now under investigation in this laboratory.

Pyrolysis of cholesterol has been shown to lead to a number of definite products. Thus Diels and Linn (9) say they have obtained, by heating to 310° C. for half an hour, 50 per cent conversion to Δ4-cholestenone, and part of the rest they designated β-cholesterol. The latter substance was shown to be a molecular compound of dihydrocholesterol (48), and *epi*-allocholesterol (10). Heating with a dehydrating agent gives rise to a variety of products, including dicholesteryl ether (25) and Δ2,4- and Δ3,5-cholestadienes. Staveley and Bergmann (39) state that Δ3,5-cholestadiene is formed by merely heating cholesteryl phosphates. Heilbron and Sexton (15), who distilled cholesterol at atmospheric pressure, obtained a considerable proportion of cholestenone, but also isolated a fairly large amount of pseudocholestene.

Of the above substances, it was easy to isolate dicholesteryl ether by reason of its ethanol insolubility, and cholestenone by extraction of the ethanol-soluble material with cold methanol; neither is carcinogenic. The residue left after removal of these two compounds is being fed to a group of rats, so far without its having induced any stomach tumors (20 months).

Neither β-cholesterol nor Δ3,5-cholestadiene has been isolated in this experiment, although the instantaneous carmine red color given by the diene with antimony trichloride in chloroform (51) was seen in some chromatogram filtrates. Nor has any pseudocholestene been obtained, though this may be due to the fact that Heilbron and Sexton must have raised their material to a much higher temperature.

In view of Waterman's assertions (50) that cholesterol becomes carcinogenic on heating by virtue of its dehydration to Δ3,5-cholestadiene and that he has obtained "precancerous" lesions in mice fed with this diene (47), it was felt desirable to feed this substance to a further group of rats; so far (18 months) no tumors have been observed. Strong (41) has shown that neither the Δ2,4- nor Δ3,5-diene is carcinogenic for

CBA mice when painted or injected by the usual technics.

Cholesterol is present in the esterified condition in natural sources and there is always the possibility that, under the influence of heat, these esters would break down by a different route. Certainly the oxidation by air of cholesterol in colloidal suspension is notably reduced in the case of esters (3). Feeding experiments are therefore being carried out in two groups of rats, one receiving cholesteryl palmitate and the other cholesteryl stearate, both heated to 300° C. for half an hour. A final group of rats is being fed cholesterol heated to 430° C. for half an hour, to determine whether a temperature approaching that at which food is charred is sufficient to produce a carcinogen.

SUMMARY

1. Cholesterol heated to 270-300° C. for half an hour in air has been fed at a level of 20 mgm. daily to albino rats up to 2 years.

2. No significant lesion, from the point of view of carcinogenesis, was observed in either part of the stomach.

3. The role of diet in the production of stomach lesions is discussed.

4. Preliminary observations regarding pyrolytic decomposition of cholesterol are recorded, including the formation of a substance having a blue fluorescence in the ultraviolet beam.

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Human Neoplasms in Tissue Culture

II. Observations upon Cells Derived from Peritoneal and Pleural Effusions*

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Both neoplastic and nonneoplastic cells are found in pleural and peritoneal effusions due to malignant neoplasms. Are these cells viable and capable of proliferation when they come to rest upon a suitable surface? More especially are neoplastic cells, floating in such fluids, still viable? If so, it would lend support to the idea that carcinomatosis of serous surfaces can result from implantation, independent of vascular and lymphatic dissemination. Also, can a colony be formed by the multiplication of a single neoplastic cell? This question is of considerable interest, as it bears upon the fundamental potentialities of neoplastic cells. These questions might be answered by studying the cellular constituents of pleural and peritoneal effusions in tissue culture.

MATERIAL AND METHODS

Pleural and peritoneal fluids were obtained through the cooperation of the staffs of the Hospital of the University of Pennsylvania and the Philadelphia General Hospital. In each instance the fluid was withdrawn aseptically, and about 10 to 15 cc. put into each of two test tubes. One of these was centrifuged and the plug of cells thus thrown down was fixed, embedded, sectioned, and stained. The other tube, destined to supply cells for culture, was centrifuged only if the suspension of cells was light. From the contents of this one, roller tube tissue cultures were made. If the cells had been packed by centrifuging, the plug of cells was removed and treated as a solid piece of tissue: It was cut into tiny fragments and handled as has been previously described (1) for preparing roller tube cultures of biopsy material. If the cells had not been packed by centrifugation, several drops of the ascitic or pleural fluid were allowed to run down the inside of the culture tube and mix with chicken plasma. The plasma, when clotted, then held the scattered cells in position. A fluid medium consisting of human fetal serum and a physiological salt solution was added, and the tube stoppered and placed in a rotator (2) housed in an incubator. When more detailed cytological studies were desired than is possible through the wall of the pyrex culture tubes, subcultures were made in hanging drop preparations.

RESULTS

By these methods cells derived from the ascitic and pleural fluids of 23 patients with a variety of diseases have been studied in tissue culture (Table I). In some instances, several samples were obtained from the same patient.

TABLE I: FLUIDS FROM WHICH CELLS WERE OBTAINED FOR TISSUE CULTURE

Source of fluid	Disease	Number of cases
Peritoneal cavity	Carcinoma of ovary	3
	Carcinoma of stomach	2
	Cardiac failure	3
	Cirrhosis of liver	3
Pleural cavities	Fibrosarcoma	2
	Metastatic carcinoma	1
	Leukemia	1
	Cardiac failure	4
	Cirrhosis of liver	4

CELLS DERIVED FROM PERITONEAL EFFUSIONS

It seems desirable to consider first those cells encountered whether or not a malignant tumor was present, since they can be expected to appear in all cases. The following types were found in peritoneal effusions from patients with cirrhosis of the liver or cardiac decompensation:

Macrophages.—These were the cells encountered most commonly. When cultures were examined immediately after preparation the macrophages appeared as scattered spherical cells, but within a few minutes after incubation they changed their shape, flattened out, formed pseudopodia, and exhibited sluggish ameboid movement. In most instances they tended to die out after a week or so of culture; less

* This investigation was aided by a grant from The International Cancer Research Foundation.

often, they continued to live and increase. When cultures consisted of fragments of plugs of centrifuged cells the macrophages sometimes were so numerous that they formed almost solid masses of outwandering cells. At times, particularly in cultures more than a few days old, interlacing networks of cells were formed (Fig. 1).

Polymorphonuclear leukocytes.—Scattered polymorphonuclear leukocytes, common in peritoneal effusions, were found in every specimen. These cells are actively ameboid and phagocytic in culture, but do not usually survive for more than a few days. No living polymorphonuclear leukocytes were encountered in cultures more than 1 week old.

Lymphocytes.—These cells were extremely numerous in some samples of fluid, almost absent in others, even when the different samples of fluid were obtained from the same patient, but upon different days. Like the polymorphonuclear leukocytes they persisted for only a short time, usually no more than a week or 10 days.

Mesothelial cells.—These large cells were frequently spherical when examined soon after the culture was prepared. After incubation they spread out and assumed irregular shapes with long filamentous processes and pseudopodia (Fig. 2). Their movement was extremely sluggish. These cells often had two or more nucleoli and sometimes more than one nucleus. They frequently formed loose networks (3).

Fibroblasts.—These elongated spindle cells were found in nearly all cultures more than a week old (Fig. 3). It seems significant that they were not encountered during the first few days of culture. This fact suggests that they might be derived from some other cell type, and raises the old question as to the possible transformation of macrophages into fibroblasts, or of mesothelial cells into fibroblasts. As yet this problem has not been thoroughly investigated by the present method, which indeed would seem to afford an excellent means of answering the question. In some cultures the fibroblasts eventually dominated the scene, producing a closely woven meshwork of spindle cells.

Macrophages, polymorphonuclear leukocytes, lymphocytes, mesothelial cells, and fibroblasts, then, can be reasonably expected to appear in all cultures prepared from peritoneal effusions.

Neoplastic cells.—Specimens of peritoneal fluid were obtained from 3 patients with ovarian carcinoma and from 2 with gastric carcinoma. Carcinoma cells were found in cultures of these fluids, in addition to the varieties described above. These cells proliferated and, as is characteristic of epithelial cells (1), formed loosely knit sheets (Fig. 4). They persisted in the cultures and showed no tendency to die out, even after many weeks of existence *in vitro*. The individual epithelial cells varied considerably in appearance. They were usually flat and polyhedral, sometimes with irregular borders. Nuclear division without cytoplasmic cleavage occasionally resulted in multinucleated giant cells. The nucleoli were usually easily visible and frequently multiple.

Isolated carcinoma cells were repeatedly observed and studied, having been scattered throughout the plasma during preparation of the cultures. Such single cells were known to be neoplastic because no other epithelial cells were present in the fluids. More or less continuous observation of such an isolated cell disclosed that it might live for a time and then die and disintegrate, or that it might divide and, by subsequent division of the daughter cells, give rise to a colony. Such an early colony is shown in Figs. 5 and 6. This observation seems to offer an interesting field for further investigation since a pure strain of neoplastic cells derived from a single cell could be obtained for study. It also seems indisputable that even a single neoplastic cell can remain viable after floating around in ascitic fluid and that it can form a colony after coming to rest upon a suitable surface.

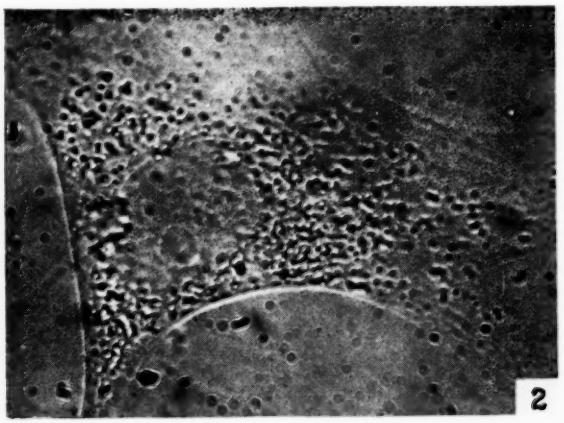
Cells Derived from Pleural Effusions

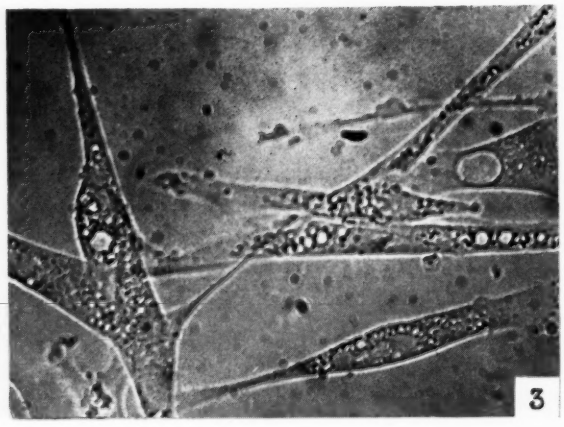
Macrophages, polymorphonuclear leukocytes, lymphocytes, mesothelial cells, and fibroblasts were found in about the same numbers as in fluid from the peritoneal cavity, and none of them showed any significant differences from those obtained from the ascitic fluids.

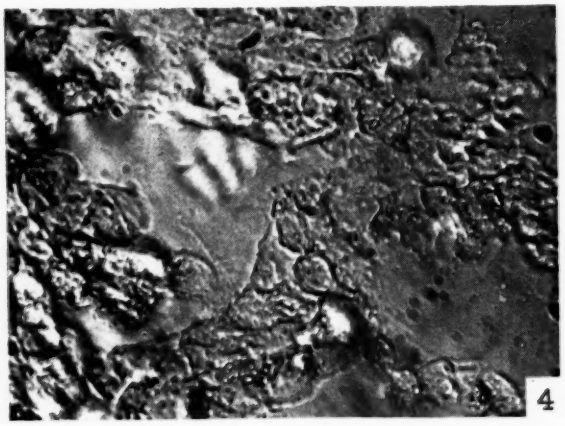
In addition, one culture yielded endothelial cells. These formed tubes resembling capillaries (Fig. 7), varying in caliber and in length and anastomosing to form a network as described by Lewis (4). A question arises as to the origin of these endothelial cells. It is conceivable that a few endothelial cells were carried away by the needle from vessels of the chest wall during the thoracentesis. No other plausible explanation of their origin has presented itself.

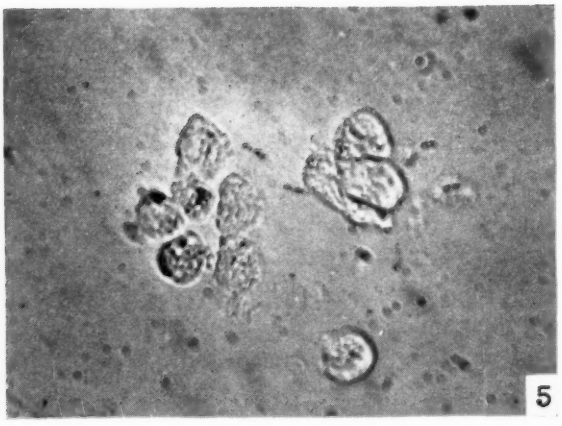
Neoplastic cells.—Cultures were made of cells in pleural fluids obtained from 2 patients with fibrosarcoma, from 1 with a metastatic carcinoma of the pleura, and from 1 with leukemia. The diagnosis in each instance was confirmed by biopsy.

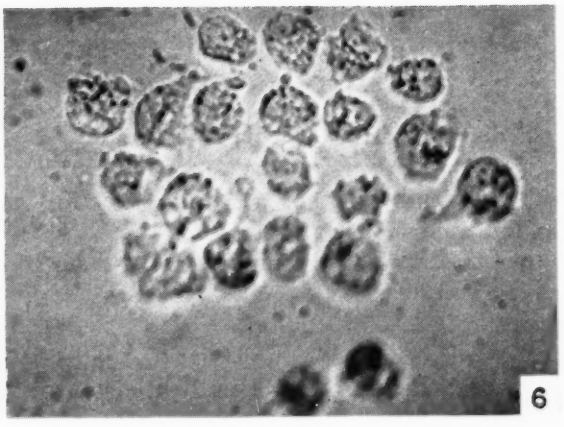
From the cases of fibrosarcoma was obtained luxuriant proliferation of malignant fibroblasts (Fig. 8). These cells grew vigorously, surviving subculture in hanging drop preparations, and continued to grow profusely in the roller tubes. When the fluid itself was examined microscopically only an occasional cell was found with an appearance suggestive of malignancy, so that in these two cases it would have been well-nigh impossible to reach a diagnosis of fibrosarcoma by the usual histological methods of examining the fluid; yet in the cultures there could be no

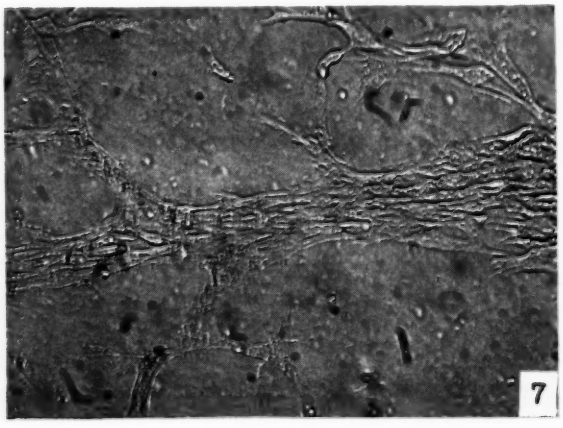


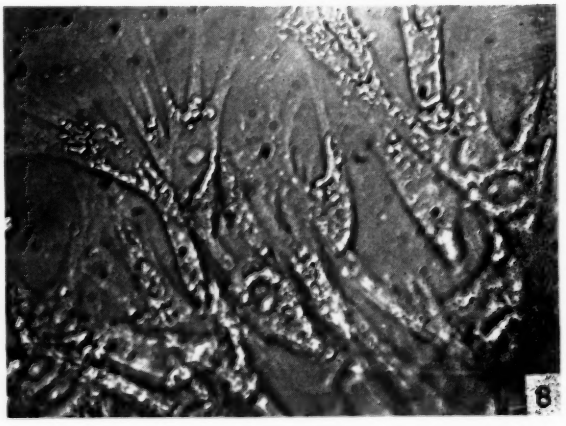












FIGS. 1-8

doubt as to the nature of the condition. The cytological characteristics of malignant fibroblasts in tissue culture have been well described by Lewis (5).

Culture of the fluid obtained from the patient with metastatic carcinoma showed the typical epithelial sheet formation similar to that described for cells obtained from peritoneal fluid. The site of the primary growth has not been determined at this time, but the sedimented cells that were fixed in formalin and stained with hematoxylin and eosin revealed clusters of epithelium suggesting an adenocarcinoma.

The fluid obtained from the leukemic patient was of a slightly pink milky character and extremely rich in cells. The predominant type found upon examination of the hematoxylin and eosin preparations was the myeloblast. In culture there was seen a profusion of these myeloid elements for a few days only. Later there was a sharp decrease in their number and a notable increase in macrophages, which 2 weeks later had formed interlacing networks over the glass surface of the culture tube.

DISCUSSION

It has been generally thought that carcinomatosis of serous membranes could be produced by implantation though, as pointed out by Sampson (6), the evidence has never been more than circumstantial. The evidence is still not conclusive, but it has here been shown that neoplastic cells that have floated about in ascitic or pleural fluid remain viable and capable of proliferation when given a suitable surface for attachment and furnished with a nutrient medium. This strongly supports the contention that metastasis by implantation can give rise to carcinomatosis of the serous membranes.

Given the proper environment, a single neoplastic cell can multiply and produce a colony. This would seem to open up an interesting field for future exploration, for by studying such cells and their colonies in tissue culture it may be possible to answer several pertinent questions. For instance, do mutations occur? How closely do all the descendants of a single cell resemble each other and the original parent cell? Are there, in the same tumor, different strains of cells varying in their degree of anaplasia?

The culture of cells derived from pleural and ascitic fluids can, in some instances at least, be of diagnostic aid. But as determination of the ultimate value of this method would require considerable time and a large group of patients it could be employed only in an institution where the services of a tissue culture laboratory were readily available.

SUMMARY AND CONCLUSIONS

Cells derived from pleural and peritoneal effusions were grown in tissue culture by the roller tube method.

Macrophages, polymorphonuclear leukocytes, lymphocytes, mesothelial cells, and fibroblasts were cultured from all fluids. In one instance endothelial cells were found, and these produced structures resembling capillaries.

Cells from carcinomas and sarcomas grew vigorously, thereby indicating that such cells remain viable and capable of proliferation after floating in pleural or peritoneal fluids if given a satisfactory surface to which they may become attached. Thus support is given to the view that carcinomatosis of serous membranes can occur by implantation.

Small colonies were observed to develop from single neoplastic cells that had become isolated in the supporting plasma of the tissue culture. The significance of this observation is discussed as opening a field for further exploration.

DESCRIPTION OF FIGURES 1 TO 8

FIG. 1.—Macrophages in roller tube tissue culture showing formation of loose network after a week of existence *in vitro*. These cells were obtained from ascitic fluid. When examined soon after the cultures were prepared the macrophages were spherical. Soon, however, they formed pseudopodia and were actively ameboid. Mag. × 110.

FIG. 2.—A single mesothelial cell in hanging drop subculture from roller tube. This cell was obtained from pleural fluid. Such cells sometimes formed loose sheets *in vitro;* when single they sent out long processes. Most of the small dark dots within the cytoplasm are mitochondria. Multiple nucleoli are a common feature of mesothelial cells. Mag. × 1050.

FIG. 3.—Fibroblasts from ascitic fluid, in hanging drop subculture from roller tube. Fibroblasts are multipolar or elongated spindle cells with thin cytoplasm, few mitochondria, and variable amounts of fat. Mag. × 480.

FIG. 4.—Carcinoma cells forming an extremely loose and irregular sheet in roller tube culture. These cells were cultured from peritoneal fluid from a patient with carcinoma of the stomach. Carcinoma cells usually grew in delicate sheets one cell thick. They had a pronounced tendency to liquefy the supporting plasma. Mag. × 400.

FIGS. 5 and 6.—Two stages in the formation of a small colony from isolated carcinoma cells in roller tube cultures. In Fig. 6 the 10 cells shown in Fig. 5 have increased by multiplication to 21 in 36 hours. Mag. × 400.

FIG. 7.—Endothelial cells in roller tube culture forming a branching capillary-like structure. These cells were cultured from ascitic fluid, but perhaps were derived from vessels of the chest wall dislocated by thoracentesis. Mag. × 110.

FIG. 8.—Fibrosarcoma cells in roller tube culture of pleural fluid. Malignant fibroblasts were frequently somewhat larger than normal fibroblasts, had a denser cytoplasm, and were often multinuclear. Commonly sarcoma cells showed larger nucleoli than normal cells and the nucleoli were often multiple. Mag. × 400.

It is suggested that the culture of cells from pleural and ascitic fluids can be of aid in diagnosis under favorable circumstances.

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Nucleolar Vacuoles in Living Normal and Malignant Fibroblasts*

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INTRODUCTION

Nucleolar vacuoles can frequently be seen in living normal and malignant fibroblasts of rats and mice in our hanging drop cultures. They are probably similar to the "nucleolar or intranucleolar vacuoles or bodies" noted by various authors in many different types of animals.

Since the origin and significance of nucleolar vacuoles is unknown, I attempted to determine if there were any correlations between their occurrence and some cultural or cytological characteristics of normal and of malignant fibroblasts. No consistent ones were found.

I also attempted to determine if there were significant differences between normal and malignant fibroblasts in cultures in regard to the number of cells with nucleolar vacuoles, the number per nucleolus, and their size. No differences were found except that an occasional sarcoma had relatively more cells with them and more vacuoles per nucleolus than were found in cultures of normal fibroblasts.

TECHNIC

All observations were made on living cells in hanging drop cultures sealed on brass rings and with 3 or 4 explants per culture. Only cells attached to the cover glass were thin enough to permit a clear view of the nucleoli. The relative number of cells with nucleolar vacuoles was estimated by a survey of the culture with a 2 mm. lens. No attempt was made to determine the exact percentage of cells with nucleolar vacuoles. Note was made whether they were absent, few, common, or many, and on the number per nucleolus and size. Observations were made at the same time on the extent of the outgrowth, relative number of mitoses, number of nuclei per cell, number of nucleoli per nucleus, condition of the nucleoplasm, amount of fat, mitochondrial content, cytoplasmic vacuoles, size of central area, pinocytosis, and general condition of the cells.

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NORMAL FIBROBLASTS FROM THE BODY WALL OF 1 DAY OLD MICE (FIGS. 2 TO 4)

From 1 to 5 series of 1 to 6 cultures each were set up at different times in chicken plasma or human cord serum and various combinations of one or both with one or more of the following: Gey's saline, Locke's solution and modification of it, beef embryo extract, and water. Altogether there were 36 series and 18 different media with a total of 152 cultures.

Nucleolar vacuoles were noted in 127 of the 152 cultures, in one or more cultures in every medium employed, and in each of the 36 series of cultures. Fibroblasts with nucleolar vacuoles were numerous or moderate in number in one or more cultures of most of the series. There were also cultures in each of the media in which no cells or only a few were noted with nucleolar vacuoles. There was thus considerable variation in the relative number of cells with nucleolar vacuoles in the different cultures with each medium but there were no significant differences between the media except that poor cultures usually had no vacuoles or only a few cells with them.

The number of vacuoles per nucleolus varied from 1 to 25. There were some nucleoli with 5 to 10 vacuoles in most of the series. As a rule, cultures with the greatest number of cells with nucleolar vacuoles had the greatest number of vacuoles per nucleolus.

There was considerable variation in the length of life of cells in different cultures, even in the same medium, due to unknown factors. Those cultures that lived the longest, 8 to 14 days, had relatively more cells with nucleolar vacuoles than those that died early, at 2 to 4 days.

Of 100 cultures followed daily until death of the cells, 11 had no nucleolar vacuoles, 48 showed very little change in the relative number of cells with nucleolar vacuoles, 22 showed an increase in the course of the first few days and then a decrease, 13 showed an increase but no decrease, and 6 showed a decrease after the first day or two. Some cultures with no vacuoles after the first day or two acquired them later and others with vacuoles the first few days lost them.

The relative number of cells with nucleolar vacuoles had apparently very little to do with the extent

of the outgrowth, unless it was poor, or with the number of mitoses or the amount of pinocytosis, or the number of fat globules or mitochondria, or any other cytological feature such as the number of nucleoli, the condition of the nucleoplasm, the accumulation of neutral red stainable vacuoles and granules, or the enlargement of the central area that usually accompanies such accumulations.

There was no particular evidence that the relative number of cells with nucleolar vacuoles increased during cell degeneration preceding their death. There were on the contrary, as already noted, often fewer nucleolar vacuoles in poor cultures that lived only 2 or 3 days than in active ones. Only 2 of 13 infected cultures had them. The outgrowths of the latter were poor and the cells died in the course of a day or two.

Young Mouse Fibroblasts from Roller Tube Cultures (Fig. 2)

Fibroblasts from the body wall of 1 to 28 day old mice were cultivated in a series of 15 roller tubes for 15 to 189 days in medium 61A, consisting of 3 parts chicken plasma plus 1 part embryo extract for the clot in which the explants were embedded and a nutrient fluid consisting of 7 parts Gey's saline plus 3 parts human placental serum plus 2 parts embryo extract. The nutrient fluid was changed every 3 or 4 days and the colonies were occasionally transferred to fresh tubes unless otherwise stated. Thirty-one hanging drop cultures were set up from the tube cultures in the same medium with all the ingredients mixed together, and 4 were set up in chicken plasma.

There was usually good migration and mitosis. Nucleolar vacuoles were found in every culture. Some cultures had a few cells with 1 to 4 vacuoles per nucleolus and some had many cells with 1 to 10 or more vacuoles per nucleolus. The majority of cultures had a moderate number of cells with 1 to 10 vacuoles per nucleolus. Some cultures that had no cells or only a few cells with nucleolar vacuoles on the first or second day had few to many cells with them on subsequent days. Some cultures showed after a few days a decrease of the relative number of cells with them, and some cultures showed no particular change.

There were no special differences between the cultures and the relative number of cells with nucleolar vacuoles that could be attributed to the age of the tube, or the age of the mouse or the chicken plasma medium, or any cultural or cytological characteristic.

Adult Rat Fibroblasts from Roller Tube Cultures (Fig. 1)

Since adult rat fibroblasts exhibited scanty and retarded migration in hanging drop cultures made from tissues taken directly from the animal, they were first cultivated in 20 roller tubes for 14 to 233 days in medium 61A with the usual changes of supernatant fluid every 3 or 4 days and occasional transfers to fresh tubes. Twenty-two series, consisting of 117 hanging drop cultures, were set up in medium 61A and one series of 11 cultures in a medium consisting of 7 parts saline plus 3 parts human placental serum plus 2 parts beef embryo extract. There were great differences in the outgrowths in hanging drop cultures from the different tubes due to the condition of the cells in the tubes at the time the cultures were made. Some of the best outgrowths were from a 231 day tube. Of 60 cultures examined, 36 had no cells with nucleolar vacuoles, 17 had a few cells with them, and 7 a moderate number of cells with 1 to 10 or more vacuoles per nucleolus.

Some cultures showed an increase, some a decrease, and some no particular change from day to day of the relative number of cells with nucleolar vacuoles. There were no consistent correlations between the number of mitoses, the age of the culture, or any special cultural or cytological features of the cells and the number of cells with nucleolar vacuoles or the number of vacuoles per nucleolus, except that the outgrowths were not as a rule as extensive as those from the young mice and the relative number of cells with nucleolar vacuoles was not as numerous. On the other hand, the idea that cells with nucleolar vacuoles are fewer because cultures are poor is somewhat nullified by the occasional entire absence of cells with nucleolar vacuoles in good cultures.

Malignant Fibroblasts from Dibenzanthracene Mouse Sarcomas (Figs. 5 to 8)

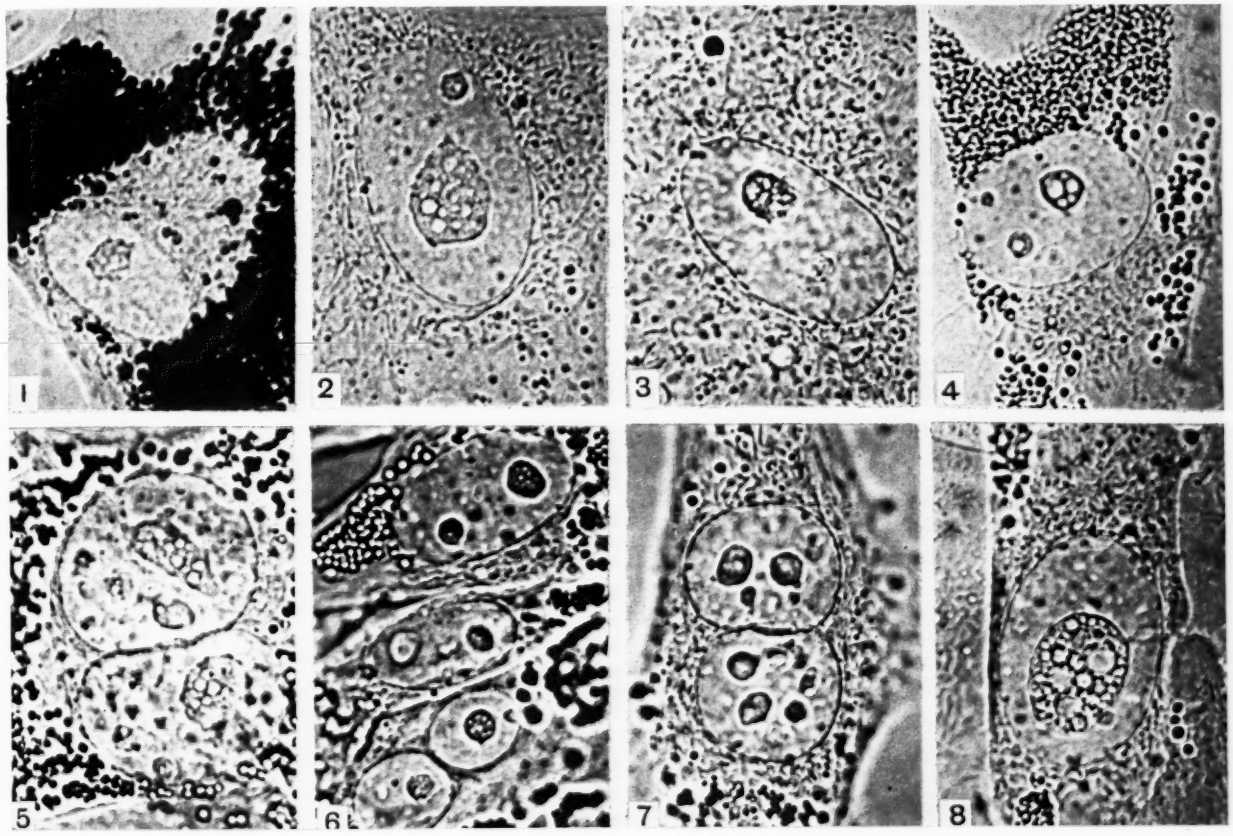
One to 7 series of hanging drop cultures were set up at different times from 34 of our induced spindle cell sarcomas, either from the primary and/or one or more passage tumors. This involved 129 series with 1 to 5 cultures each, a total of 271 cultures. Two media were usually employed, namely: (a) 1 part chicken plasma plus 1 part beef embryo extract and (b) No. 61A, 7 parts saline plus 3 parts human placental serum plus 2 parts beef embryo extract plus 3 parts chicken plasma.

Cultures from different passages of the same tumor differed about as much in regard to the relative number of cells with nucleolar vacuoles as did cultures from the same passage.

The tumors can be arranged in a graded series in respect to the relative number of malignant fibroblasts with nucleolar vacuoles. At one end of the series there were 4 that had no vacuoles in some cultures and only a few cells in others with them. A second group of 11 tumors had no vacuoles or only a few cells with

them in most of the cultures, but in an occasional culture cells with nucleolar vacuoles were common or many. In a third group of 9 tumors, cells with nucleolar vacuoles were usually common but occasional cultures had either few or many. A fourth group of 8 tumors generally had many cells with nucleolar vacuoles but there were occasional cultures with few vacuoles. A fifth group of 2 tumors had nucleolar vacuoles in many of the cells in all the cultures. There were 2 cultures in this group with nucleolar vacuoles in every cell.

As a rule, the number of vacuoles per nucleolus varied directly with the number of cells containing them. Cultures that had few cells with vacuoles had 1 to 5, occasionally 8 per nucleolus. Cultures with many cells with nucleolar vacuoles had from 1 to many small and occasionally an additional one or two large vacuoles per nucleolus.



Nuclear areas of normal and malignant fibroblasts. × 1,100 diameters.

FIG. 1.—Adult rat. Two day hanging drop culture from a 55 day tube culture, both in medium 61A except that the hanging drop culture had neutral red. Broad zone of deeply staining neutral red bodies (black in photograph), large central area with many mitochondria. Irregular nucleolus has 12 small unstained vacuoles.

FIG. 2.—One day old mouse. Six day hanging drop culture from a 13 day roller tube culture, both in medium 61A. Few small fat globules, many mitochondria. Large nucleolus has 19 vacuoles, small nucleolus 2 vacuoles.

FIG. 3.—One day old mouse. Six day culture, 4 parts saline plus 3 parts human placental serum plus 2 parts beef embryo extract. Many mitochondria, no fat. Twelve small nucleolar vacuoles.

FIG. 4.—One day old mouse. Five day culture in chicken plasma. Many fat globules. Three nucleoli, the largest with 6 vacuoles, the medium sized nucleolus with one.

FIG. 5.—Binucleate malignant cell, from dibenzanthracene mouse sarcoma, 4th animal passage. One day culture in 1 part chicken plasma plus 1 part beef embryo extract. Zone of fat globules surrounding nuclei. Nucleolar vacuoles in both nuclei.

FIG. 6.—Malignant cells from another dibenzanthracene mouse sarcoma, 5th animal passage. Two day culture in chicken plasma and embryo extract. Many minute nucleolar vacuoles.

FIG. 7.—Binucleate malignant cell from another dibenzanthracene mouse sarcoma. Five day culture in chicken plasma and embryo extract after 9 animal passages, 120 days in roller tube culture, then 12 more animal passages. Some fat globules and many mitochondria. One nucleolus has 1 well defined vacuole and 2 pale areas, other nucleoli have pale areas some of which are probably thin areas or refraction phenomena.

FIG. 8.—Malignant fibroblast from another dibenzanthracene mouse sarcoma. One day hanging drop culture in chicken plasma-serum-extract medium from a tube culture after 15 animal passages and 31 days in tube culture in medium 61A. The large nucleolus has about 50 vacuoles that differ somewhat in size.

The relative number of cells with nucleolar vacuoles varied in most cultures from day to day. Some cultures with no vacuoles or only a few on the first or second days had more on the third or fourth days, some cultures did not show such changes, and some showed a decrease. There was usually an increase or a decrease in the number of vacuoles per nucleolus with the corresponding increase or decrease in the relative number of cells with them.

There was no consistent correlation between the relative number of cells with nucleolar vacuoles and the medium, the extent of migration, the number of mitoses, or any particular cultural or cytological feature, except that the malignant fibroblasts from some tumors that flattened out on the cover glass more than those from others, had as a rule more nucleolar vacuoles than the compact spindle-shaped cells from other tumors.

Several hundred photographs ($\times$1,100 diameters) of living malignant cells in cultures of 184 additional dibenzanthracene mouse sarcomas, usually the primary and/or one or more passage tumors, were taken to show the typical malignant cells of each tumor without consideration of the presence or absence of nucleolar vacuoles. Cells from 132 of the tumors show nucleolar vacuoles and those from 52 do not. Some of the latter would probably have shown cells with nucleolar vacuoles if cultures had been made of more passage tumors. Among the 132 tumors with nucleolar vacuoles there were many that did not have nucleolar vacuoles in the photographs of cells from one or more of the passage tumors.

Induced Rat Sarcomas

One hundred and eighty series of hanging drop cultures from 55 rat fibrosarcomas produced by dibenzanthracene, methylcholanthrene, or benzpyrene were set up in chicken plasma and in medium 61A and sometimes also in chicken plasma plus rat serum. Cultures were made from the primary and/or one or more passage tumors. Usually only the best culture of each series was examined. There were no particular differences between the dibenzanthracene, methylcholanthrene, and benzyprene tumors or the medium used in the cultures as regards the relative number of cells with nucleolar vacuoles.

No nucleolar vacuoles were found in any of the cultures from 9 of the tumors. Some of the cultures from 23 of the tumors had a few cells with them and some had no vacuoles. Nucleolar vacuoles were common in some of the cultures from 16 of the tumors, yet there were few or none in the other cultures from them. Some of the cultures from 7 of the tumors had many cells with nucleolar vacuoles and some had only a few or no cells with them.

There were 1 to 4 vacuoles per nucleolus when relatively few cells had them and 1 to many when there were many cells with them. The number of vacuoles also varied with the size of the nucleoli, which were often larger than normal.

Almost every culture had some cells with many small granular nucleoli instead of a few large ones. The granules were either concentrated in the central part of the nucleus or more or less scattered. Cultures from some tumors had many cells with them and those from other tumors had only a few. No vacuoles were seen in such small nucleoli.

Among many photographs at 1,200 diameters of the typical malignant cells of 54 of the rat sarcomas mentioned above, 519 with 623 cells show the nucleoli clearly enough for their vacuolar content to be determined. No especial attention was given to the presence or absence of nucleolar vacuoles at the time the photographs were made. Only 185 of the cells had nucleolar vacuoles. The photographs show about the same distribution of nucleolar vacuoles as do the direct observations. Nucleolar vacuoles are absent in photographs of cells of 11 of the tumors, absent to few in 24 tumors, absent to common in 10 tumors, and absent to many in 9 tumors.

Spontaneous Rat Tumors

Eight tubes of the spontaneous Crocker rat tumor 10 were carried for 81 to 85 days in a fluid medium consisting of 7 parts Gey's saline plus 3 parts human placental serum plus 2 parts beef embryo extract, with the usual changes of nutrient medium every 3 or 4 days and an occasional transfer to a fresh tube. Sixteen hanging drop cultures were set up in medium 61A. The outgrowths had radii of 0.5 to 1.5 mm. Ten cultures had no cells with nucleolar vacuoles, 5 had a few cells with 1 to 6 vacuoles per nucleolus, and 1 had an increase from a few to a moderate number of cells with 3 to 7 or more vacuoles per nucleolus. When first found many years ago this tumor was a carcinoma; after repeated transfers it assumed a somewhat sarcomatous aspect but is probably a much modified carcinoma. Every culture had some cells showing fragmentation of their nuclei into 2 to 25 segments, often unequal in size. Most cultures had cells with cytoplasmic vacuoles, pinocytosis, and mitosis.

Hanging drop cultures from another tube carried for 141 days in medium 61A with 3 per cent alcohol had poor outgrowths and no cells with nucleolar vacuoles.

Ten tubes of the spontaneous Walker rat sarcoma 319 were carried for 16 to 133 days in medium 61A, with the usual changes of nutrient fluid and occasional transfers. Twenty-three hanging drop cultures were set up in the same medium. Outgrowths had

radii of 0.5 to 1.0 mm. Mitosis and pinocytosis were noted in most of the cultures. Twenty cultures had no cells with vacuoles, 1 had a few, and 2 had a moderate number of cells with 1 to 3 vacuoles per nucleolus.

Numerous photographs of the malignant cells in cultures from 11 other rat tumors, 1 spontaneous carcinoma and 10 sarcomas (cysticercus and spontaneous), show more cells without than with nucleolar vacuoles.

General Considerations

Nucleoli are probably semisolid bodies. This suggestion is substantiated by the fact that irregular nucleoli retain their distinctive forms for several days even when the nucleus becomes temporarily distorted by changes in cell shape. The nucleoplasm is probably also in the gel state. This is substantiated by the fact that when several nucleoli are present they retain their spatial relations to one another even after temporary distortions of the nucleus. The nucleolar pattern (number, size, shape, and location) in any given nucleus remains fairly constant for long periods, perhaps from one cell division to the next when mitoses are frequent.

Many nucleolar vacuoles were spherical, others were somewhat oval. They contained clear fluid that did not stain with neutral red (Fig. 1). Occasional large centrally located ones appeared to exert pressure on and distort peripherally located ones. There were indications that some fused to form larger ones.

In addition to the clearly defined vacuoles pale areas were frequently seen both in the living nucleoli and in the photographs of them. Some were due to local differences in the thickness of irregular nucleoli but others appeared to be due to changes within nucleoli which made small areas less opaque. The latter may have been forerunners of vacuoles.

Normal fibroblasts had 1 to 30 and malignant ones 1 to 60 vacuoles per nucleolus. When cells with vacuoles were numerous the number per nucleolus was usually increased and varied roughly with the size of the nucleolus. When relatively few cells had them there were but one to a few in even the largest nucleoli. When vacuoles were numerous all the nucleoli in a cell except the granular ones, if present, usually had them. This also applies to binucleate and multinucleate cells. When vacuoles were few not all the nucleoli in some cells had them.

It was evident from day to day observations that vacuoles sometimes increased in number or decreased and disappeared in the course of a few days. Presumably vacuoles may also increase or decrease in size from day to day.

Measurements from photographs of 1,100 and 1,200 diameters give less than 0.5 to about 5 microns for nucleolar vacuole diameters; diameters of 3 to 5 microns were rare. The most usual size was from less than 0.5 to 1 micron. There was no particular difference between normal and malignant fibroblasts as regards size. Some nucleoli had only small ones and some had vacuoles of different sizes. The largest ones were almost always accompanied by smaller vacuoles.

Nucleoli are compound bodies formed in young daughter nuclei by the agglutination of several small nucleolar units. Normal and malignant fibroblasts ordinarily have from 1 to 8 nucleoli.

Occasional normal fibroblasts and more commonly malignant ones, especially from some tumors, have many granular nucleoli. This may indicate nonagglutination or a later splitting, perhaps by nucleolar vacuoles, into the granular or nucleolar units. I have not been able to find vacuoles in the granular units. It seems probable that the vacuoles develop between these units.

Nucleoli are probably organs with a specific function and nucleolar vacuoles may represent metabolic products that ordinarily diffuse out into the nucleus but for unknown reasons are prevented temporarily from doing so.

DISCUSSION

Page, Regan, and MacCarty[1] have reviewed the literature and described nucleolar vacuoles in stained fresh frozen and fixed sections of normal tissues and of benign and malignant tumors of the human subject. The following quotations show that their findings differ somewhat from mine. "In normal tissues they occur infrequently and we have never seen more than one in a nucleolus." Fibroblasts in my cultures frequently had vacuoles and there were often more than 2 per nucleolus. "In malignant cells, refractive bodies are large and easily seen. They are present in many cells per field, and from one to eight or more may be seen in each nucleolus." Malignant fibroblasts from some of our tumors had no vacuoles and only a few tumors had many cells with them. The technics employed and the types of tissues and tumors examined probably account for the differences in our findings.

SUMMARY

1. Many hundred cultures of living normal and malignant fibroblasts from rat and mouse tissues were cultivated in various media and examined for nucleolar vacuoles.

2. Some cultures of normal fibroblasts had no nucleolar vacuoles, some had a few or a moderate number, and some had many cells with them.

3. Malignant fibroblasts from some tumors had no nucleolar vacuoles, those from others had a few or a

[1] Page, R. C., Regan, J. F., and MacCarty, W. C. Intranucleolar Bodies in Normal and Neoplastic Human Tissue. Am. J. Cancer, **32**:383-394. 1938.

moderate number, and those from a few tumors had many cells with them.

4. No consistent correlations were found in either normal or malignant cells between the number of cells with nucleolar vacuoles and the culture medium, the extent of migration, the life of the culture, the number of mitoses, the amount of pinocytosis, or any cytological feature such as the number and size of the nucleoli, the condition of the nucleoplasm, the number of nuclei, the number of fat globules, the mitochondria, the neutral red staining vacuoles and granules, and the size of the central area.

5. Normal fibroblasts had 1 to 30 and malignant ones 1 to 60 vacuoles per nucleolus. The number of vacuoles per nucleolus usually varied directly with the number of cells that had them and with the size of the nucleolus.

6. The relative number of cells with nucleolar vacuoles may increase or decrease during the life of a culture.

7. Malignant fibroblasts cannot as a rule be distinguished from normal ones by the relative number of cells with nucleolar vacuoles, by the number of vacuoles per nucleolus, or by the size of the vacuoles.

Yolk Sac Cultivation of Tumors

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INTRODUCTION

Some months ago there was published from this laboratory a brief preliminary report of a method whereby tumors could be cultivated in the yolk sacs of developing chick embryos (4). Neoplasms were first injected into the yolk sac with the idea that the tumor cells would die and disintegrate, thus releasing any virus that might be present. It was hoped that if such a release occurred the medium possibly might favor the growth and concentration of the tumor-producing principle, as Cox and others have found for the viruses of Rocky Mountain spotted fever and typhus groups (1). Since publication of the preliminary results, this technic has been used here extensively in studies involving large numbers of eggs, and it is now felt that a further and more detailed description of the method would be of value.

Special interest has been focused on the use of this medium for growing tumors because it was found possible to produce malignant neoplasms in mice with cell-free extracts of the yolk material from eggs implanted with cancer (3). The technic was hit upon in the course of investigations leading up to the discovery of this virus-like principle.

It occasioned considerable surprise when eggs so treated were opened and well developed tumors were discovered in the yolk sacs. The principles involved are essentially the same as those discovered by Murphy when he found that the chick chorioallantoic membrane and the chick itself were capable of supporting the growth of tumor tissue from the mouse and the rat (2). In the present instance the location of the growth is different, but in both methods the chick supports the cancer. In the yolk sac method the tumor does not interfere with the chick and inoculation is relatively simple.

Eggs Used

Eggs are obtained from a flock of pure bred white Leghorn hens. All our egg requirements have been satisfied from this same source for the past two years. The chickens are maintained on a well balanced diet. Egg fertility during the fall, winter, and spring seasons is over 90 per cent. The embryos are healthy and very few of them die after the fifth day of incubation has been reached successfully. However, other eggs have been used, including ordinary "yard eggs," without notable loss in utility for growing tumor material.

Incubation

The main incubator is a 1,200 egg cabinet fitted with a motor-driven fan and apparatus for the regulation of temperature and humidity. It holds 8 separate trays, enabling the operator to stagger the incubation periods of groups of eggs so that eggs are available for injection at spaced intervals—daily if desired. In addition, several all-metal, round, 100 egg electric machines are available for special work. The majority of the eggs are run at the normal temperature for chick-hatching—37.6° C.

Tumor Tissue

Both mouse and rat tumors have been cultured, either directly from the animal host or indirectly by transplants from other eggs. Several lines of spontaneous and transplanted mouse tumors, and a carcinosarcoma of the rat, Walker 256, have been grown without difficulty. No trouble has been encountered with any tumor whose growth rate is sufficient to produce a sizable mass within the incubation period of the chick. In the usual routine, tumors are used for inoculation before any appreciable necrosis has developed.

Preparation of the Tumor for Injection

A tumor-bearing animal is lightly etherized and then decapitated. After bleeding, the body is immersed briefly in a solution of 70 per cent alcohol to which a little iodine has been added. This treatment makes it simpler to remove the tumor without contamination from the pelage. The tumor is grown in the caudal region of the animal so that it can be exposed easily by incising the skin completely around the body just posterior to the forelimbs and then rolling it backward. Until recently the excised tumor has been placed in a piece of coarse, unbleached, domestic muslin, the edges of the cloth have been folded

over, and the tumor tissue has been squeezed through the cloth by twisting the pouch thus formed. In this manner the soft parts of the tissue can be dispersed so that they will easily pass through a 20 gauge hypodermic needle, while the tougher connective tissue elements remain behind in the sack. The paste so obtained is placed immediately in a 5 cc. syringe and then injected through the rubber stopper of a 30 cc. serum bottle (Fig. 1). Rigid asepsis is maintained at every step of the procedure.

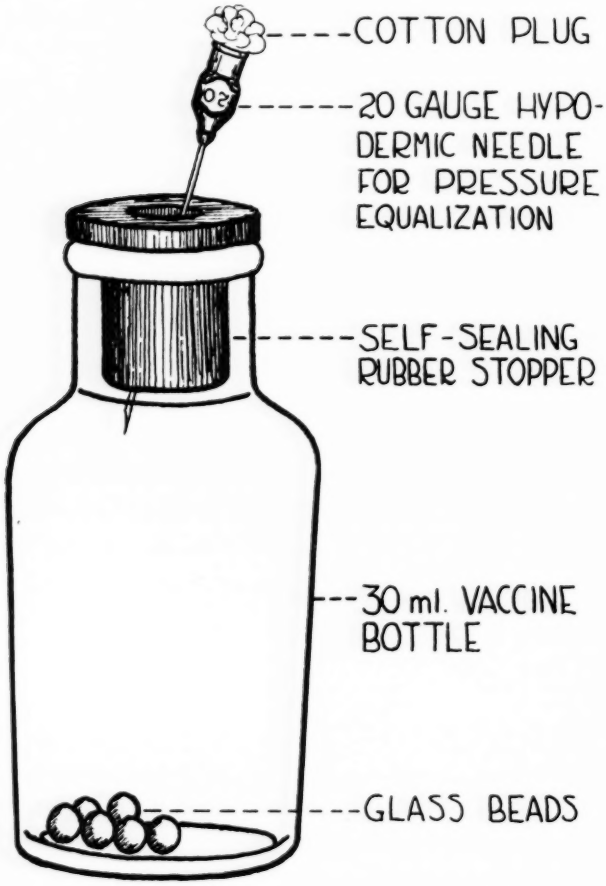

FIG. 1.—Serum bottle for reception of dispersed tumor tissue.

Recently an alternative method has been used with success on both mouse and rat tumors. Wire screens of suitable mesh are placed in the barrel of a 10 ml. Luer syringe, separated by glass beads. The plunger is inserted and the syringe is dry sterilized. The tissue to be injected is removed from the host and placed in the syringe, and the mass is forced through the screens into the barrel of a second syringe without screens, in which it is measured and diluted according to requirements. The screens hold back the more resistant tissues but permit easy passage of the cancer cells. Iron wire screens with 18 meshes to the inch have been found to yield a suspension that readily passes through a 20 gauge needle. The mesh and needle size may be varied as desired.

When the tumor for injection is taken from a previously implanted yolk sac it is not necessary to squeeze the material through the cloth. Yolk sac-grown tumors are much softer than those grown in animals so that the tissue can be placed immediately in the syringe. It may then be forced without difficulty through a 20 gauge needle into the serum bottle.

The practice has been to suspend 1 volume of tumor paste in 4 volumes of an 0.85 per cent saline solution and to use 0.5 cc. of the resultant mixture, containing 0.1 cc. of tumor for each egg injected. However, individual tumors may require different treatment in this respect.

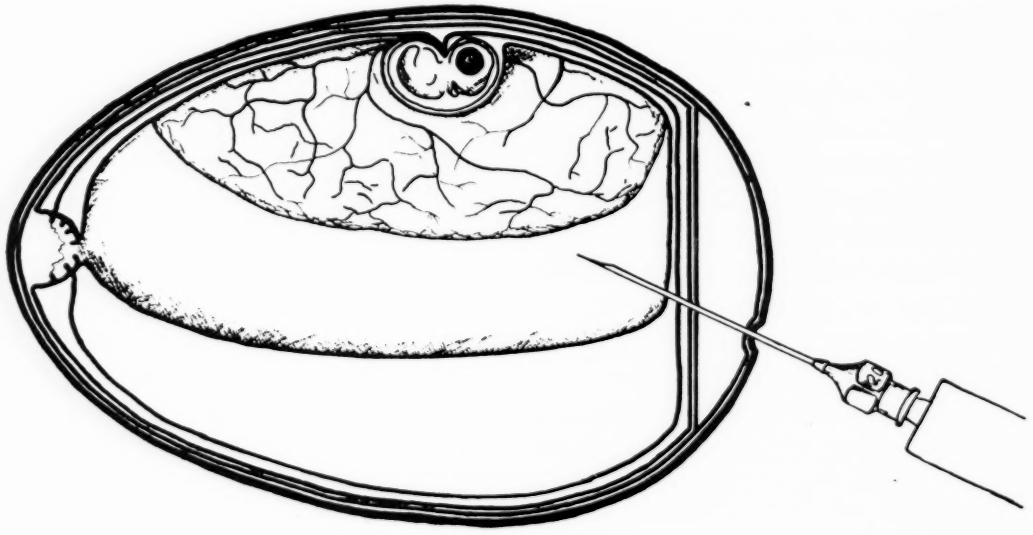

FIG. 2.—Diagrammatic representation of the egg after 5 days' incubation. Hypodermic needle is in place for yolk sac inoculation.

FIG. 3.—Chick and yolk sac with tumor after 17 days of incubation.

FIG. 4.—Chick and 4 gm. tumor that has been removed from the yolk sac.

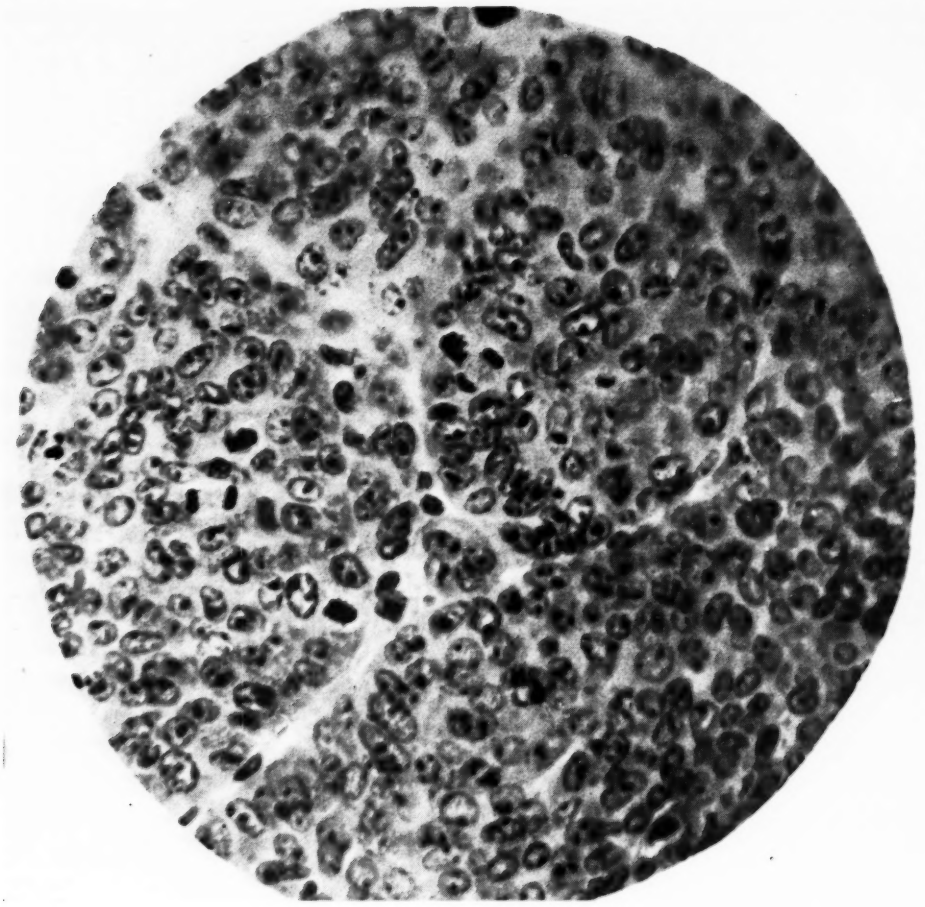

FIG. 5.—The dba transplantable carcinoma that has been used in the greater part of the egg work. Mag. × 1,000.

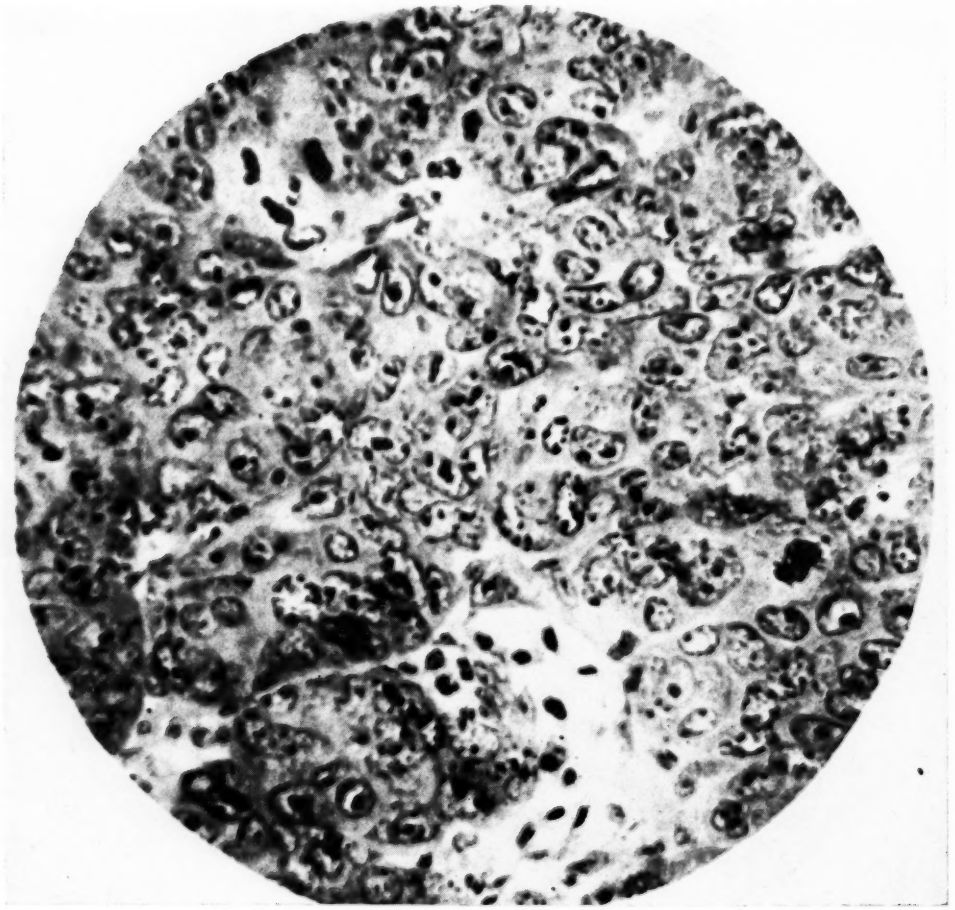

FIG. 6.—The tumor shown in Fig. 5 as it appears when growing in the yolk sac of the chick embryo. Mag. × 1,000.

Yolk Sac Implantation

In preparation for injection, eggs that have been incubated for about 5 days are allowed to remain at least 30 minutes without turning. Since the eggs are incubated while resting in a horizontal rather than an upright position, as is the practice followed in some experimental work, the embryo floats to the top of the yolk just above and posterior to the air space. Taking care not to change the position the egg has maintained for the previous half hour or so, the operator sterilizes the surface of the shell covering the air space and taps a small hole to permit entrance of the syringe needle. One point of a medium sized scissors may be used for this operation. The shell is pierced, but not the inner shell membrane.

The saline suspension of tumor is injected into the yolk by means of a 1¼ inch 20 gauge hypodermic needle. The yolk is so situated that almost immediately past the air space, the needle enters the yolk sac (Fig. 2).

Many variations in the technic of implanting have been and are being tried. For example, injections have been made through the side of the egg with good results, for although a certain amount of the white oozes out the subsequent development of the embryo seems normal.

Fig. 2 illustrates the general procedure involved in the inoculation of the yolk sac. After injection, the small hole in the blunt end of the shell is sealed with cellulose tape.

Yolk Sac Tumors

The difficulty of maintaining rigid asepsis throughout the several steps necessary in transferring tumor from the mouse to the yolk sac causes the loss of a small percentage of the eggs. If massive infection occurs it is immediately evident and kills the embryos in a very short time. Light contamination may not appear for several days after implantation. Some infective organisms take considerable time to develop in the incubating egg so that as much as a week or 10 days may elapse before the death of the embryo is brought about.

When the inoculated egg is opened, at the end of 17 or 18 days of incubation, the tumor in the yolk sac tends to resemble in gross appearance those grown from the same donor tissue in host animals (Figs. 3 and 4). Histological examination discloses that the yolk sac tumor has a supporting stroma supplied by chick tissue, otherwise the general characteristics found in the donor material are preserved (Figs. 5 and 6).

Usually there is one main aggregation of neoplastic tissue situated in that region of the yolk sac known as the yolk sac umbilicus. This area, in the 17 to 18 day chick, is connected with the albumen sac. Occasionally smaller nodules of tumor are found in other regions of the yolk sac.

The chick embryo will usually live on to the termination of the incubation time even when the yolk sac contains a tumor weighing as much as 5 gm. Often the larger tumors are hemorrhagic and, as a result, the yolk sac may be filled with partially hemolyzed blood. When this happens the chick, although it may be undersized, will be fairly vigorous. Occasionally a large tumor may cause the death of the embryo a day or two before the termination of the experiment.

When the method of inoculation used is consistent for a series of eggs the resulting yolk sac tumors show distinctly less variation in size than tumors produced by implants in mice. This is to be expected when it is recalled that the mice may be resistant to a varying degree, according to their heredity, whereas the chick presumably does not contain any factors that resist heterologous cancer tissue.

The eggs of some types of fowl have a longer incubation period than hen's eggs, and the extended hatching period should make them suitable for the cultivation of comparatively slow-growing tumors. The egg of the Muscovy duck, for example, hatches after about 35 days of incubation. A few experiments with duck eggs have shown that tumors will grow in the yolk sac of these embryos.

SUMMARY

A method is described whereby tumors from the mouse and rat can be cultivated in the yolk sac of the developing chick embryo.

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The Effect of Yolk Sac-Cultivated Tumors on the Hemoglobin Level in the Embryonic Chick

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INTRODUCTION

It has long been noted by many investigators that the latter stages of cancer are usually accompanied by a more or less severe drop in the hemoglobin level. Most writers have assumed that this depression is a secondary result of general body debilitation, starvation, and hemorrhage (1, 4, 12). Weil and many others (8, 11, 20) have suggested that the chief factor may be some type of hemolytic agent or agents liberated into the blood stream by the necrosing and ulcerating areas of closed tumors, a theory also agreeing with the consensus that the hemoglobin depression is a secondary chlorotic effect, for it presumes that only necrotic or dying tumor can release the hemolysins.

In human beings this effect has not been proved to be a universal result of cancer. Blood has been found to be abnormally concentrated in the very advanced stages of cancer in some patients with hemoglobin values up to 100 per cent at death (7). A good many case histories have been recorded of persons whose hemoglobin levels were in no way affected by a developing malignant tumor (7). Thus the over-all picture of the association of anemia with human cancer is not as clear cut as might be desired. The difficulty has been, of course, that where man is the subject of observation it is impossible to conduct a controlled investigation, and clinical investigators have been forced to gather their data from patients with diverse types of cancer and widely varying individual susceptibilities and resistances.

The work with lower animals has clarified the problem to some extent and has at least established the depression of hemoglobin as a consistent by-product of certain types of malignant neoplasms. Tumor-bearing rats, mice, rabbits, and chickens, have been studied by Adliwankina and others (1, 2, 3, 5, 13), to mention a few, and in all such animals the characteristic anemia has been found in the advanced stages of tumor growth. In these researches it has been possible to control both the type of cancer and the genetic constitution of the host (5, 16, 17). But although the use of experimental animals has brought about a stabilization of the problem, the original conception of the clinicians has been preserved; *i. e.*, the depression of the hemoglobin level is a secondary effect of the animal's bodily reaction to the malignant growth.

Recently Taylor and Pollack conducted a series of investigations with both rats and mice in which they found evidence to support a new concept of the relationship between the anemia and neoplastic growth (19). They concluded that the blood deterioration is a direct and primary effect resulting from the action of a factor or factors released by the normally growing tumor and operating not only in its late necrotic stages but at its very inception and throughout its course. A definite drop in the hemoglobin level was found in precancerous stages induced by methylcholanthrene in mice and by *p*-dimethylaminoazobenzene in rats. This initial depression became further aggravated when the tumor developed, and the effect increased in severity in steady relationship to the growth of the tumor.

The present investigation is an extension of the work mentioned above. Recently a new technic was developed in which mammary carcinomas of the mouse were grown in the yolk sacs of developing chicks (18). Out of contemplation of this work arose the idea that perhaps these tumors, growing outside the chick proper but supported by the same vascular system, might have an effect upon the hemoglobin similar to that found in animals serving as hosts for neoplasms in the usual manner.

MATERIALS AND METHODS

The eggs, from a pure strain of white Leghorns, were injected on the fifth day of incubation with finely divided tumor suspended in saline solution, according to a method that has been previously described (18).

The tumors for injection were mammary carcinomas of dba mice that had been stabilized by many generations of transplantation.

The injected eggs were incubated for a total of 17

to 18 days, including the initial 5 days previous to the injection of the cancer. The controls (normal, non-injected eggs) were incubated for the same length of time and under identical conditions of temperature and humidity. The possible effect of the technical manipulation upon the hemoglobin level was checked by injecting a number of eggs with sterile saline solution. No significant deviation from the normal resulted from this procedure.

When the 17th to 18th day of incubation had been reached, the chicks were removed from the shell and other membranes, and the blood for hemoglobin determinations was taken directly from the omphalomesenteric vein. The determination of the hemoglobin was accomplished by the use of Evelyn's photoelectric colorimeter according to the method described by him (6). Erythrocyte counts also were made on blood from specimens of the control and experimental groups.

The tumors were dissected out of the yolk sacs and weighed. They usually consisted of more or less discrete masses, but not infrequently examination with a binocular dissecting microscope disclosed very small nodules of tumor scattered over the yolk membrane. It was decided not to attempt the tedious procedure of collecting all these as they represented an exceedingly small percentage of the tumor present and so could exert but little influence upon the *trend* which the present experiment sought to demonstrate.

Chicks from some of the tumor eggs were found to have ruptured yolk sac membranes that allowed the yolk to intermingle with the white. These were rejected, as were all those possessed of any discernible peculiarity such as discoloration of the yolk material, etc.

RESULTS

Effect on Hemoglobin

The hemoglobin level in chicks supporting the mouse carcinoma in their yolk sacs was depressed considerably below the level of the controls. In fact the larger tumors caused hemoglobin decreases in excess of 50 per cent. This is shown clearly in Table I and Fig. 1. The severity of this phenomenon increased, with minor irregularities, in direct relation to the size of the tumor supported by the developing chick. The hemoglobin index tended to be low (0.73), which conforms with the majority of the available data on human cancer patients (7).

Effect on the Chick

The gross anatomy of the chick appeared to be unchanged. There were no deformities of any part of the body, but tumor-bearing chicks did tend to be smaller than the controls. The body weight was found to be less when the tumor weight was greater. This was to be expected since the chick is dependent upon a food supply that is constant in quantity, and any superfluous growth that draws upon and diminishes this supply leaves so much less for the embryo. The average weight of the normal 17 to 18 day chicks was approximately 16 gm., whereas the mean weight of 10 chicks supporting tumors averaging 1.7 gm. was 12.86 gm. On the other hand, the mean weight of 10 chicks whose tumors averaged only 0.48 gm. was 14.2 gm. Tumor chicks that were allowed to develop completely and hatch appeared to be healthy and normal in every particular except size. The visceral organs were apparently in normal condition with the exception of the liver, which had become paler and of a blotchy appearance. The spleen weight seemed to be slightly increased—about 5 to 10 per cent.

Table I

Source of data	Number of chicks	Age, days	Tumor weight, gm.	Hemoglobin, gm. per 100 cc.	Relative hemoglobin values
Controls	30	17–18	—	6.75 ± 0.77	100.00
Experimental					
No. 1	6	17–18	0.03 ± 0.02	5.79 ± 0.43	85.75
No. 2	10	17–18	0.10 ± 0.00	5.51 ± 0.90	81.60
No. 3	7	17–18	0.18 ± 0.04	4.28 ± 1.16	63.40
No. 4	7	17–18	0.33 ± 0.08	4.30 ± 0.53	63.70
No. 5	6	17–18	0.84 ± 0.11	4.39 ± 1.14	65.00
No. 6	15	17–18	1.87 ± 0.50	3.20 ± 1.22	47.40

Appearance of the Tumors

The nodules varied somewhat in appearance. Some were composed of fairly solid lumps of tissue while others were softer and tended to fall apart more readily upon dissection. A little less than 20 per cent were more or less hemorrhagic. Histologically these tumors resembled the original transplanted carcinomas grown in the mouse, but the supporting tissues were supplied by the chick. Microscopic examination further revealed that they were young, healthy neoplasms with no indications of necrosis.

DISCUSSION

The results leave no doubt that the growth of the foreign malignant tumors in the yolk sacs of embryonic chicks induced a decided fall in hemoglobin values. While this effect was greatest when the tumor had attained a weight of a gram or more, the drop of 19.4 per cent effected by growths weighing only 0.1 gm. is sufficiently pronounced to be of more than statistical significance.

Aside from a decrease in size the chicks themselves appeared to be relatively unaffected by the growth of the tumors. This was indicated by the fact that those supporting sizable cancers in their yolk sacs were successfully carried through to the post-hatching period; if the tumors were extremely large, however, the chicks were not always able to survive until hatching time. Therefore it seems unlikely that the entire depressant effect upon the hemoglobin level was merely the result of secondary conditions. Moreover, although some of the larger tumors were hemorrhagic, this seemed not to play an over important part in the total effect. For example, a chick with a hemorrhagic tumor weighing 1.67 gm. had a hemoglobin value of 2.06 gm. per 100 cc.; another, incubated in the same group, possessed a nonhemorrhagic tumor weighing 1.64 gm. and had a hemoglobin value also of exactly 2.06 gm. per 100 cc.

In view of the direct relationship between hemoglobin level and tumor size it may be presumed that the tumor cells produce a substance or substances which, when liberated into the blood stream, exert in some manner an inhibitory influence upon the blood hemoglobin concentration. This effect, of course, could be produced either by erythrocyte destruction, or through interference with the functioning of the hemopoietic system, or by a combination of these methods. In this connection it may be worth pointing out that the livers of the tumor-bearing chicks became somewhat altered in appearance, as was mentioned in the results. The liver was the only organ that gave any obvious evidence of abnormality; and since during a portion of the chick's embryonic period that organ assists in maintaining the quality of the blood, it seems possible that interference with its proper function may have been a contributing cause in the general hemoglobin depression. A converse relationship was observed by Murphy, who transplanted bone marrow and spleen from adult chickens into embryonic chicks supporting rat sarcomas in their fused chorioallantoic membranes and brought about regression of the tumor tissue (9, 10). Later investigations, however, have failed to verify these results completely (14, 15).

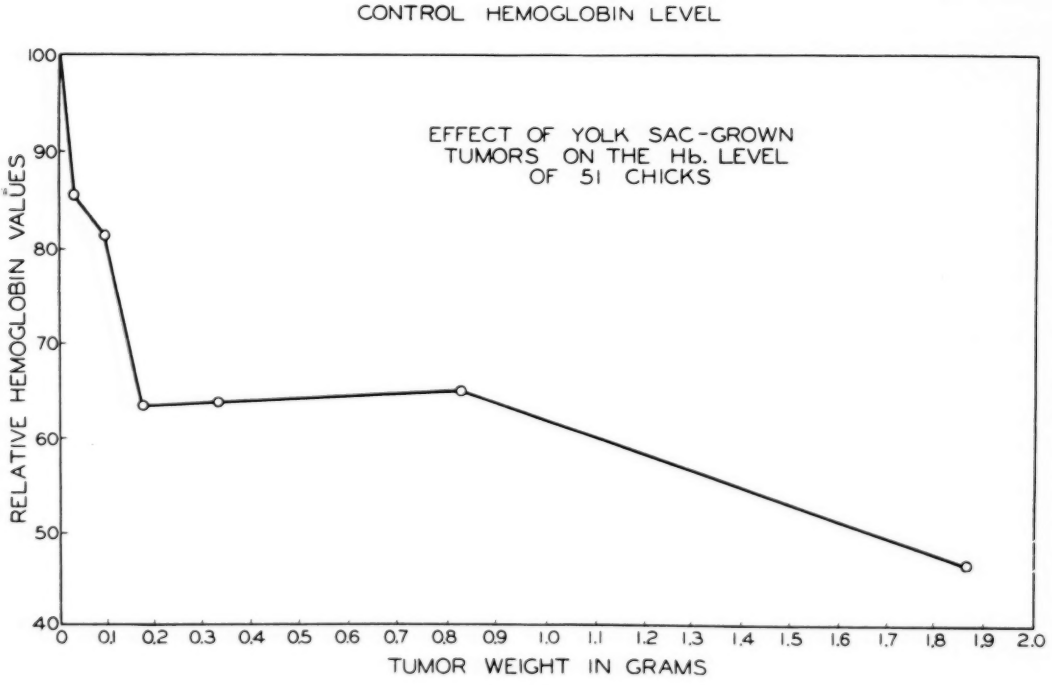

FIG. 1

SUMMARY

Mammary carcinomas of mice were grown in the yolk sacs of 51 embryonic white Leghorn chicks. The hemoglobin level of these chicks was found to be considerably depressed by the 17th to 18th day of incubation (as much as 70 per cent in individual cases).

The severity of the depressant action was in a direct and fairly constant relation to the size of the tumor. A diminution in the size of the chick and possible damage to the liver were the only other apparent results of the procedure.

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The Growth of Alien Strain Tumors in Parabiotic Mice*

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The method of parabiosis has been called into use by a number of workers in efforts to determine whether resistance to transplanted tumors is conditional on the presence of circulating antibodies in the host. In 1909 Rous (13) united tumor-bearing rats with those of another strain which were resistant to the growth of the tumor, but this procedure did not produce any observable effect on the growth of the tumors. Lambert (8), however, asserted that mouse sarcoma grew better and for a longer time in rats joined parabiotically with mice than in intact rats. According to Albrecht and Hecht (1), the parabiotic relation in itself led to a resistance against the transplanted tumors; mouse carcinoma grafted into either member of a pair of mice grew much more slowly than in controls. Similar results were reported in rats by Kross (7), who preferred to base an explanation on possible ill health of the animals. The illuminating researches of Morpurgo (11) tended to confirm the earlier conclusions of Rous. Morpurgo found that if susceptible and resistant rats were united and the tumor concerned placed in the peritoneal cavity common to the two animals, growth of the implanted cells occurred only in the tissues of the susceptible animal. Recently Furth, Barnes, and Brower (5) have used the parabiotic technic to investigate whether susceptibility or resistance to leukemia in mice is transferable. In varied series, involving the union of susceptible with resistant mice, no evidence was found that either of these hereditary qualities could be modified by parabiosis.

In all the investigations mentioned above the animals termed resistant had either acquired resistance prior to operation or possessed this condition as a hereditary trait. It is of additional interest to study the development of resistance in animals already in parabiotic connection, and in particular, the influence of regression of a tumor in one parabiont on the later inoculation of the same tumor in the opposite parabiont. With the development of pure inbred strains of mice and refined methods of inducing malignant growths, mouse tumors are now available that will grow temporarily in alien strains of mice but later regress, leaving the hosts 100 per cent immune from subsequent transplants of the same tumor (9). In the present experiments these have been utilized to produce immunity in parabiotic mice of alien strains, and to test for the spread of such resistance between members of a pair.

MATERIALS

Mice of three inbred strains, A (Andervont), BA (Bagg albino), and C57 black were used, in each of which tumors were at hand for experimental work. These were sarcomas A274, BA1, C57-241, induced by subcutaneous inoculation of 1,2,5,6-dibenzanthracene and maintained by serial transplantation. M. R. Lewis (9) has described the growth behavior and immunity induced by these neoplasms on inoculation into alien strains of mice. Of the three, BA1 grows consistently in A or C57 mice to form a moderate sized tumor, which later invariably regresses. Subsequent transplants of BA1 in the same animals never show any growth. C57-241 nearly always grows to a fairly large size on inoculation into A or BA mice. Its growth energy is variable, and it may continue to grow progressively in the alien host or it may regress and disappear. In either event the mouse is immune from repeated inoculation of C57-241. The third sarcoma, A274, differs in that it usually grows progressively in both BA and C57 mice, and this process does not always confer an immunity on the host against later inoculations of the tumor. The differing growth characteristics of these three tumors will be of importance in interpreting the experimental results.

METHODS

Parabiotic union of the mice was accomplished surgically under ether anesthesia. The method of Sauerbruch and Heyde (14), as modified by Bunster and Meyer (2), was followed throughout. In brief, this

* This investigation was aided by a grant to Dr. Warren H. Lewis from The International Cancer Research Foundation.

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technic involves joining the two animals laterally by means of a skin suture from the level of the hind limbs to the back of the head. Internally, the abdominal wall is incised on the inner side of each animal and all four cut edges drawn together with a single continuous suture. In this way, a firm, extensive union of the two abdominal walls is obtained without, however, uniting the two peritoneal cavities (Fig. 1). With the use of pure inbred strains of mice, no apparent advantage was found in uniting mice from the same litter or of the same sex. In all pairs, however, both mice were normal animals of the same strain.

Tumor material was inoculated subcutaneously with a trocar. Aseptic precautions were observed in removing and injecting it, and only fresh, healthy portions

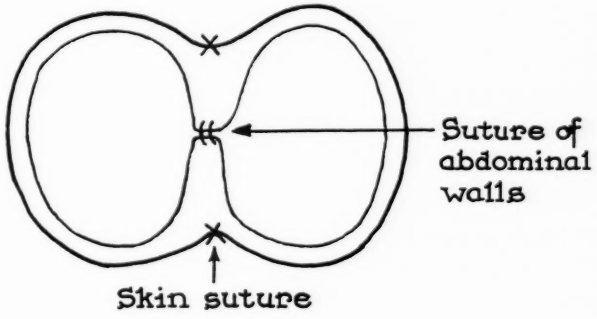

FIG. 1

were used. As controls, single mice of the same strain as the parabiotic pair were inoculated in all experiments.

The parabiotic procedure is apparently a drastic one and a physiologic incompatibility subsequently develops in many pairs, the causes for which are as yet poorly understood (3, 10). In addition, the animals are subject to infection to a greater degree than in ordinary operations, and may be unmatched temperamentally. Because of these factors a relatively high mortality was experienced in the present experiments, as in those reported by other workers. The results to be discussed here, however, are based only on pairs that survived the 2 month experimental period in good health; all other animals were discarded.

RESULTS

Healing was variable, but usually fairly complete in 2 or 3 weeks. Typically, the skin of the two parabionts fused completely along the line of junction, leaving only a faint scar. In some pairs the anterior sutures tore free, but this condition could usually be controlled with a strip of adhesive tape around the inner forelegs of the animals. In harmonious parabiotic twins, of the type used for the experiments to be described, there was no outward sign of incompatibility over the experimental period and the nutrition of the parabionts was approximately normal. Tumor inoculation was not begun until at least 25 days after operation. By this time healing was practically complete, and an extensive connection between the two mice well established.

The series of inoculations of BA1 tumor into parabiotic twins yielded the most uniform results, and will be described first. In this group were included 11 C57 × C57 and 5 A × A pairs of mice. Each of these pairs received 2 tumor inoculations, spaced approximately 10 days apart. In the first injection, made 25 to 30 days after operation, a subcutaneous dose of tumor was placed in the outer flank of the left parabiont. In all animals this primary inoculation gave rise after a week or 10 days to a tumor measuring on the average about 7 by 7 by 10 mm. Within another 2 weeks these tumors had completely regressed and were no longer palpable. The second injection, made 10 days after the primary inoculation, consisted of a similar dose of tumor into the outer flank of the right parabiont. Single control mice of the same strain as the injected pair were also inoculated with the tumor. In contrast to the vigorous proliferation in controls, tumor inoculated into the right parabionts did not in any instance give rise to definite growth. About a week after the second injection, a slight lump, approximately 1 by 2 by 5 mm. was present at the site of inoculation. This mass disappeared within another week or two and probably represented reaction by the host for the most part, rather than true growth. Three pairs of mice were sacrificed one week after the second inoculation, and the tumor in the right parabiont was compared with autopsied normal controls that had been inoculated at the same time. Whereas the tumor fragments were well vascularized and actively enlarging in control mice, in the parabiotic animals there was no significant disturbance of the normal host vascular pattern near the tumor mass. The pieces of tumor were pale, avascular, and showed no apparent increase in size. The general picture of the tumor in such parabiotic mice coincided with that following injection of BA1 tumor into normal A or C57 mice that were immune from previous inoculation.

The failure of BA1 tumor to grow in the right parabionts could not be attributed to possible ill health of the animals. Several of these C57 × C57 pairs were further inoculated subsequently with A274 and vigorous growth ensued in all cases. Other C57 pairs were finally inoculated with the homologous tumor C57-241, which grew progressively at the same rate as in controls. Hence the failure of BA1 tumor to grow in the right hand member of the parabiotic pairs was apparently correlated with the previous inoculation of

BA1 tumor into the left hand member, and was not due to some general nonspecific effect.

A lesser number of pairs, 8 BA × BA and 2 A × A combinations, were given similar staggered injections of C57-241. The course of events in these animals, except for a few differences to be mentioned, followed the outline given above. In one pair the primary inoculation, for undetermined reasons, did not give rise to any apparent growth. All other primary injections were followed by vigorous proliferation of the implanted cells. In some animals the tumor fragments, inoculated into the left parabiont 25 days after operation came to form a fairly large tumor, which eventually regressed and disappeared. Similar injections in other animals led to progressive tumor growth and the eventual death of the host. Regardless of whether the primary tumor in the left parabiont regressed or grew progressively, the inoculation of C57-241 tumor into the right parabiont, 10 days after the first injection, was not followed by any perceptible growth. A tiny mass, barely palpable and gradually receding, was the only evidence of inoculation. In one pair, however, the second injection resulted in a progressively growing tumor. It is significant that this was the same pair in which the primary inoculation had failed to take.

Only 3 parabiotic pairs, C57 × C57, were doubly injected with A274, and the results correspondingly lack statistical significance. They are nevertheless of considerable interest. The tumor in all 3 cases grew progressively as a primary inoculation in the left parabiont. After injection 10 days later into the right parabiont, progressive growth again occurred in 2 animals, with a negative result in the third. This finding correlates well with the known behavior of the tumor in control C57 mice, where a single inoculation does not always bring about an immunity from repeated inoculation.

The results of these experiments are summarized in Table I. While the number of animals in some categories is small, the results taken together are obviously significant. From these data it is clear that immunity develops in both parabionts after inoculation of one member of a pair with an appropriate tumor. The uninjected parabiont acquires the immune condition even though the tumor cells concerned were never present in the body.

TABLE I: GROWTH OF ALIEN STRAIN TUMORS IN PARABIOTIC MICE

Description of pair	Tumor used	Growth in left parabiont as primary tumor	Growth in right parabiont, inoculated 10 days later	Growth in control mice of same strain
C57 × C57	BA1	x x x x x x x x x x x	0 0 0 0 0 0 0 0 0 0 0	x x x x x x x x x x x x
A × A	BA1	x x x x x	0 0 0 0 0	x x x x x x x x x x x x
BA × BA	C57-241	x x + + + + + 0	0 0 0 0 0 0 0 +	x x x + + + 0 0
A × A	C57-241	+ x	0 0	+ + + + x 0
C57 × C57	A274	+ + +	0 + +	+ +

Each symbol refers to an individual animal. + = Progressive growth of tumor; x = vigorous growth of tumor followed later by complete regression; 0 = no growth of inoculated tumor.

DISCUSSION

The degree of communication between members of a pair is a critical factor in all experiments involving the parabiotic technic. Early workers such as Friedberger and Nasetti (4) and Ranzi and Ehrlich (12) demonstrated that bacteria or bacterial antibodies placed in one parabiont were soon distributed between both members of a pair; more recently Hill (6) showed with parabiotic rats that dye injected into one animal reached equal concentrations in both partners within 6 hours. Until recently, however, it was uncertain whether blood vessels of the two parabionts were in actual anastomosis. This point has been clarified through the work of Furth, Barnes, and Brower (5) on parabiotic mice. Rat erythrocytes, injected intravenously into one mouse, could be detected by agglutination tests in serum from the opposite partner. Similarly, nucleated chicken erythrocytes injected intravenously into one animal were demonstrable later in histologic sections of organs from the other parabiont. Hence it may be assumed that a certain degree of direct continuity exists between the circulatory systems of parabiotic animals.

The results of the present experiments suggest that immunity from alien strain tumors depends on a distribution through the blood stream of products from the implanted cells. It is difficult on any other basis to explain the development of resistance in both para-

biotic animals, when only one is inoculated with a tumor. One possible explanation would assume that tumor cells in an alien environment give off characteristic biochemical factors that in the case of parabiotic animals circulate through both partners *via* the connected vascular systems. These factors, probably protein in nature, could call forth resistance simultaneously in both members of a pair. It is also possible that antibodies against the implanted cells are formed by the parabiont bearing the tumor, and later spread into the opposite animal, which would thus be passively immunized. At the present time this view may not be dismissed directly but is considered less probable, since the results of Rous, of Morpurgo, and of Furth, Barnes, and Brower, mentioned above, show that susceptible animals do not become resistant through parabiotic union with an immune animal.

SUMMARY

1. Parabiotic mice have been used to study the development of immunity from alien strain mouse tumors and the spread of resistance between members of a pair. Mice of the same strain were united and inoculated with alien tumors known to induce an immunity in the hosts.

2. Primary injection of tumor into the left parabionts, 25 days after operation, resulted in a rapid proliferation of the implanted fragments. The eventual size of these tumors did not differ from that attained in single control mice of the same strain.

3. Inoculation of the same tumor 10 days later into the opposite parabionts was not followed by any perceptible growth of the implanted cells. Such fragments were not vascularized by the hosts, which appeared to be immune from the tumor.

4. It is suggested that inoculation of an immunizing tumor into one parabiont leads to simultaneous development of resistance in both animals. The mechanism of this process may involve chemical factors that are given off by the implanted tumor cells and circulate through the connected vascular systems.

I wish to express sincere appreciation to Dr. E. B. Krumbhaar and Dr. and Mrs. Warren H. Lewis for constructive suggestions and material assistance in the present work.—AUTHOR.

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Vitamin C and Tumor Growth*

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The association of vitamin C with tissue growth and physiologic activity raises the question of its relative importance in the growth and maintenance of neoplastic cells. A review of the previously reported observations on this subject has been made by Minor and Ramirez in connection with their studies in the utilization of vitamin C by cancer patients. They found that in 7 normal persons, in 1 patient with localized cancer, and in 5 with metastatic cancer the average daily utilization was 67 mgm., 68 mgm., and 125 mgm., respectively. One interpretation of this study is that neoplastic cells require larger amounts of vitamin C than nonneoplastic cells in order to maintain their higher rate of multiplication. Woodward observed a material resembling ascorbic acid present in higher concentration in transplantable rat tumors than in the normal tissues. Neither the concentration of this in the tumors nor their growth was affected by long continued injections of ascorbic acid. Sure, Theis, and Harrelson observed an appreciable decrease in the ascorbic acid content of adrenal, spleen, thymus, liver, kidney, and lung in rats after implantation and growth of the Walker carcinosarcoma 256.

In a study to determine whether or not desoxycorticosterone would inhibit the growth of the transplantable malignant mouse melanoma S39, as suggested by the observation of Hamilton that this substance specifically injured chick melanoblasts in tissue culture, vitamin C was injected into mice bearing the tumor as one control for observations on the effect of administering desoxycorticosterone. Vitamin C was employed as a control because of its alleged inhibitory effect upon melanin formation. In the first series of mice 3 of 4 receiving the vitamin and bearing the melanoma S39 exhibited a considerably

TABLE I: GROWTH OF TUMORS IN ANIMALS RECEIVING VITAMIN C AS COMPARED TO GROWTH IN CONTROLS

Experiments	Number of animals	Tumor	Injections	Results
1	4 (dba mice)	Melanosarcoma S39	10, 25 mgm. vitamin C in 22 days	Decidedly increased growth in 3 of mice receiving vitamin C
(III)	3 " "	" "	6, 50 units prolactin in 22 days	
	4 " "	" "	0	
2	6 " "	" "	11, 25 mgm. vitamin C in 26 days	Decidedly increased size of tumors in injected animals (Fig. 1)
(VII)	5 " "	" "	0	
3	2 " "	" "	3, 50 mgm., and 6, 25 mgm., in 20 days	No appreciable difference in tumor size
(VIII)	2 " "	" "	0	
4	5 " "	" "	6, 30 mgm. vitamin C in 21 days	Controls average larger than injected animals (Fig. 2)
(XIII)	6 " "	" "	0	
5	6 rats (Wistar)	Watts sarcoma	17, 50 mgm. vitamin C in 27 days	Growth of tumors slightly more rapid in injected rats
	6 " "	" "	0	
6	5 (dba mice)	Sarcoma 180	2, 40 mgm., and 2, 20 mgm., vitamin C in 7 days	All tumors grew very rapidly. No difference in size
(1)	5 " "	" "		
7	4 " "	" "	3, 20 mgm. vitamin C in 7 days	Appreciably more rapid growth in injected animals (Fig. 3)
(2)	4 " "			
8	6 (C57 black mice)	" "	3, 25 mgm., and 4, 35 mgm., vitamin C in 11 days	No essential difference in size of tumors
(3)	7 " " "			
9	6 " " "	" "	4, 30 mgm. vitamin C in 11 days	No difference in rapidity of growth (Fig. 4)
(4)	6 " " "	" "		

* This investigation was conducted under a grant from the O. C. Miller Fund for Cancer Research, The University of Chicago.

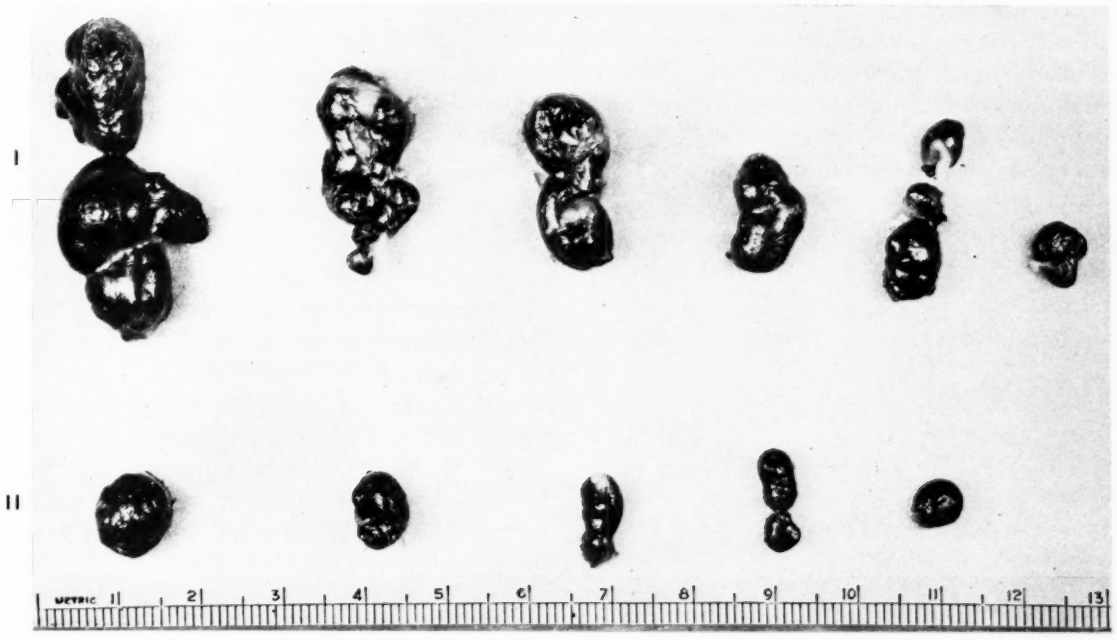

FIG. 1.—Experiment 2. (I) Melanosarcomas (S39) .from mice receiving subcutaneous injections of vitamin C, compared with tumors in control group (II). Duration of experiment 26 days.

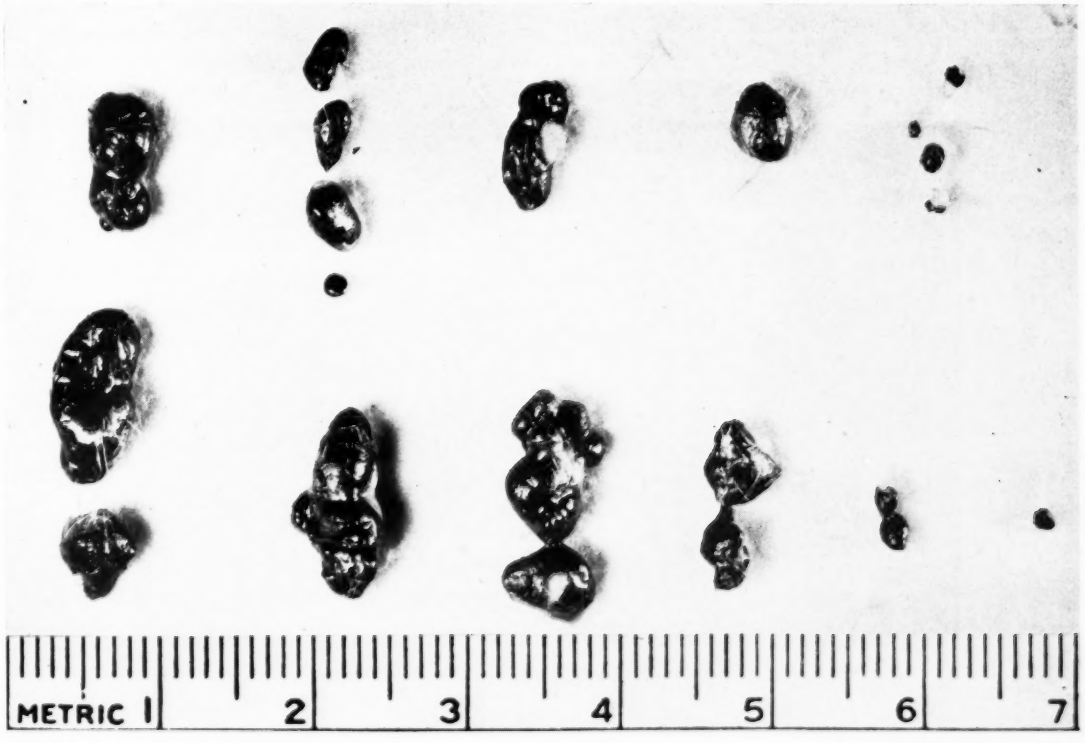

FIG. 2.—Experiment 4. Tumors (S39 melanoma) in upper row, from animals receiving vitamin C, are smaller than those in lower row, from controls. Duration of experiment 21 days. Results are the reverse of those obtained in majority of experiments (see Table I).

increased growth of the tumor. This led to further studies, which are summarized in Table I.

Experiments were conducted with only small groups of animals at a time. The animals were inoculated with the transplantable tumor, care being taken to employ small fragments of uniform size, and injections of vitamin begun 1 to 5 days afterward. The vitamin C preparation employed was received in solution in sealed ampoules containing 100 mgm. ascorbic acid in 2 cc. water (Cenolate-Abbott). Prior to the experiments all animals were maintained for some weeks on a uniform diet of dog biscuit, lettuce, carrots, and white bread.

The results of the experiments are summarized in Table I. Small differences in size were not considered significant. Photographic records were made of the excised tumors from each experiment.

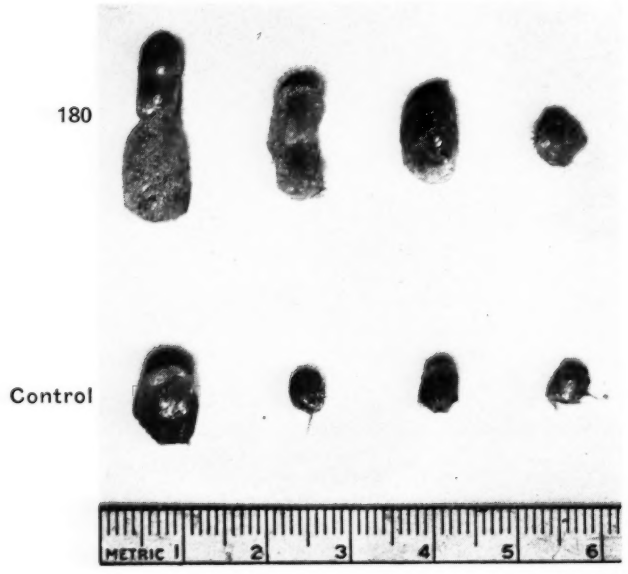

FIG. 3.—Experiment 7. More rapid growth of sarcoma 180 in mice receiving subcutaneous injections of vitamin C as compared with control group. Duration of experiment 7 days.

DISCUSSION

As the mouse and rat are able to synthesize vitamin C, the vitamin injected in the experiments described above represents an increase over that produced by the animals themselves. The results were not uniform. In experiments 1, 2, and 7 there was appreciably more rapid growth in the tumors borne by animals receiving vitamin C (Figs. 1 to 3). In experiment 5, with rats and the Watts sarcoma, growth appeared to be favored slightly by injection of the vitamin. In experiments 3, 6, 8, and 9 there was little significant difference in growth rate in the tumor in animals receiving the vitamin as compared with controls (Fig. 4). In a single experiment, No. 4, the controls grew more rapidly. It would thus appear that an excess of vitamin C in animals capable of synthesizing this substance may on occasion increase the growth rate of a transplantable tumor, but that the phenomenon is not always demontrable. That such a stimulatory action does obtain is further suggested by the fact that in none of the experiments recorded, except one, was there as decided an increase in tumor growth rate in the controls over the injected animals as was obtained in some of the groups of injected animals over the controls. Indeed, the results showed either definitely more rapid tumor growth in injected

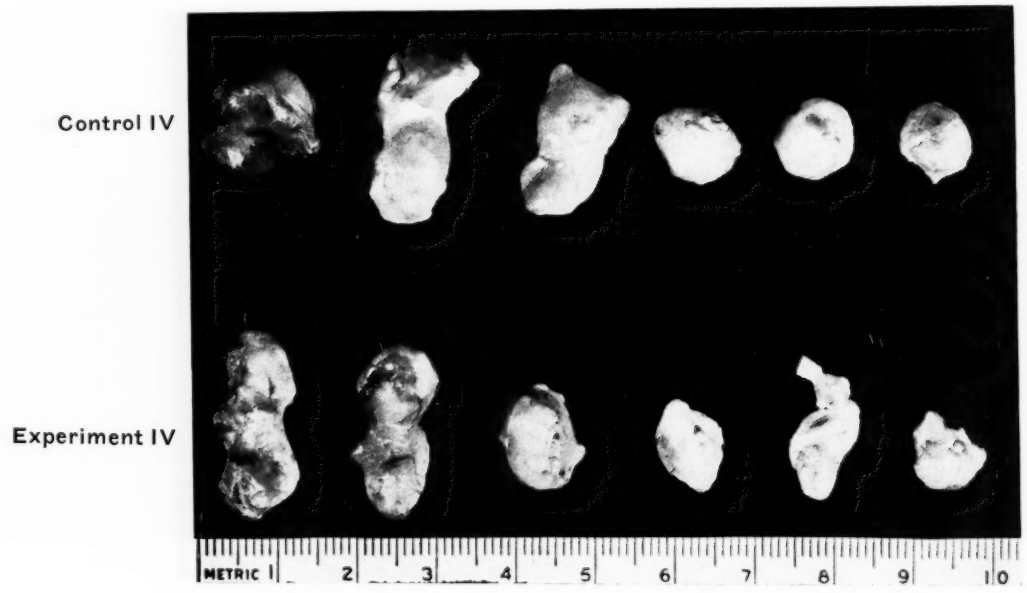

FIG. 4.—Experiment 9. No difference in the rate of tumor growth between animals receiving vitamin C (experiment IV) and controls (control IV). Sarcoma 180. Duration of experiment 11 days.

animals or no appreciable difference. The explanation for the irregularity of the stimulatory effects noted is not apparent. At any rate, though the experiments were conducted over a period from June to the following January the positive results were not limited to a particular season.

Other observers have reported a definite stimulatory effect of vitamin C upon tumor growth. Thus Fodor and Kunos found that in mice bearing the Ehrlich transplantable carcinoma, tumor growth was considerably increased if ascorbic acid was injected subcutaneously or fed in relatively high concentration by mouth. These conclusions are based upon the average weight of 10 tumors in each of 4 experimental groups compared with the average weight of 10 in each of 2 control groups. A review of their data reveals that some of the individual weights of tumors in the experimental groups were lower than some of those in the control groups. Hence the stimulatory effect was by no means uniform if it obtained at all in some instances, results that coincide with those observed in the studies reported in detail above. Vogelaar and Ehrlichman reported that in tissue cultures of mouse sarcoma 180 (Crocker) vitamin C added to the medium appeared to stimulate cell division and migration and retard cell degeneration.

As might be expected vitamin C is utilized by tumor cells as well as normal cells, but the systems in which it takes part apparently are not concerned with the fundamental nature of a neoplastic as contrasted with a nonneoplastic state. Using guinea pigs, which cannot synthesize vitamin C, Watson observed that a transplantable tumor of this species lost vitamin C when the animals were placed on a scorbutic diet, as did the normal tissues. Restoration to optimal values in the tumors demanded larger doses of ascorbic acid than was necessary for resumption of growth and repair in teeth and ribs. The vitamin C reserves in the tissues of guinea pigs on a scorbutic diet were more rapidly exhausted if the animals supported a rapidly growing tumor. Since tumor tissue grows more rapidly than mature adult tissue, it might be expected that the rapidly growing tissues would be favored as regards the vitamin. Watson also observed that the guinea pigs with growing transplantable tumors and on a scorbutic diet survived for much briefer periods than controls on a similar diet without tumors.

SUMMARY

An excess of vitamin C appears to stimulate mildly the growth of certain transplantable tumors. This stimulatory action cannot be constantly elicited under apparently uniform experimental conditions.

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Abstracts

Experimental Research, Animal Tumors

Production of Subcutaneous Sarcomas in Mice with Tars Extracted from Atmospheric Dusts. Leiter, J., Shimkin, M. B., and Shear, M. J. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:155-165. 1942.

Nine specimens of atmospheric dust were obtained from various locations by three methods of collection. Subcutaneous injection, into male C3H mice, of 20 mgm. of unextracted dust (6 specimens) in saline, or of 0.25 cc. of tricaprylin alone, did not produce sarcomas at the site of injection in 12 months. A single subcutaneous injection of about 50 mgm. of tar, extracted from these dusts by benzene and by ethyl ether and dispersed in tricaprylin, produced sarcomas at the site of injection in 18 of an effective total of 291 mice (strain C3H males and strain A mice of both sexes) in 12 months.—F. L. H.

Production of Tumors in Mice with Tars from City Dusts. Leiter, J., and Shear, M. J. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:167-174. 1942.

A new type of dust collector and two others provided specimens of black dust from various cities. Extraction of these dusts with benzene yielded black tars which were then dispersed in tricaprylin and injected subcutaneously into male mice of the C3H strain. A single injection of about 50 mgm. of tar dispersed in 0.25 cc. of tricaprylin was given. The first sarcoma appeared at the site of injection after 5 months. By the end of 16 months the incidence of sarcomas was 8%.—F. L. H.

Adenocarcinoma of the Pyloric Stomach and Other Gastric Neoplasms in Mice Induced with Carcinogenic Hydrocarbons. Stewart, H. L., and Lorenz, E. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:175-189. 1942.

The carcinogenic hydrocarbons, 20-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, were introduced by specified technics into the wall of the pyloric stomach of mice of both sexes of strains C3H, A, C, C57 black, I, and dilute brown. At autopsy the following tumors of the stomach were found: involving the glandular portion of the stomach, adenoma, adenocarcinoma, adenoacanthoma, mixed adenocarcinoma and sarcoma, mixed adenoacanthoma and sarcoma, and sarcoma; in the forestomach, squamous papilloma and squamous carcinoma.

Adenocarcinoma of the glandular stomach was induced in 6 male strain C3H mice that received 0.8 mgm. of methylcholanthrene dispersed in horse serum and in 2 male strain I mice that received 0.6 mgm. of methylcholanthrene in the same vehicle and in addition 0.14 mgm. of methylcholanthrene in mineral oil. These tumors, which were found at autopsy from 19 to 46 weeks after the time of injection, extended through the muscularis of the stomach wall and infiltrated the peritoneum. Extragastric extension and metastases were not observed. The adenocarcinoma has been successfully transplanted subcutaneously.

Extragastric extension and metastases to the liver, pancreas, mesentery, or peritoneum occurred in the case of the other malignant tumors.—F. L. H.

Intestinal Adenocarcinoma and Intra-Abdominal Hemangio-Endothelioma in Mice Ingesting Methylcholanthrene. White, J., and Stewart, H. L. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:331-347. 1942.

Mice of the C3H and C strains were fed methylcholanthrene (55 mgm. per 100 gm. of diet) incorporated in the diet for 6 months, after which time they were placed on the basal diet without methylcholanthrene. Each mouse ingested an average of 1 mgm. of methylcholanthrene per day. The mice were autopsied from 42 to 289 days after the beginning of the experiment.

Precancerous epithelial lesions and adenocarcinoma occurred in the small intestine; the intestinal carcinomas metastasized to the mesentery, mesenteric lymph nodes, pancreas, liver, and lung. Hemangioendotheliomas involving, singly or in combination, the intestine, mesentery, pancreas, or pancreatic lymph nodes developed and metastasized to the liver and the lung.

Pulmonary tumors were induced in both strains of mice, with the higher incidence in the strain C mice.—F. L. H.

Action of 5,9,10-Trimethyl-1,2-Benzanthracene on the Skin of the Mouse. Hartwell, J. L., and Stewart, H. L. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:277-285. 1942.

One hundred and twenty-four mice of the inbred strains dba, C57 black, and I, and of the first generation hybrids derived by reciprocal matings of the dba and C57 black strains, were painted on the skin of the back twice weekly with a 0.06% benzene solution of 5,9,10-trimethyl-1,2-benzanthracene. Of these, 102 were suitable for pathologic study.

The first papillomas appeared at 39 days in 2 strain I mice. Seventy-nine animals developed malignant tumors as follows: squamous cell carcinoma, 77; fibrosarcoma, 10; mixed carcinosarcoma, 3. Squamous cell carcinomatous metastases to the lymph node or lung occurred in 21 mice.

Pigmented foci in the painted area occurred in 61 mice. None of these lesions was considered to be a definite neoplasm. Their morphologic characteristics, nature, and significance are described and discussed.—Authors' summary.

Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

Influence of Limited Application of Methylcholanthrene upon Epidermal Iron and Ascorbic Acid. Carruthers, C., and Suntzeff, V. [*Barnard Free Skin and Cancer Hosp., St. Louis, Mo.*] J. Nat. Cancer Inst., **3**:217-220. 1942.

A single cutaneous application of a 0.6% (weight/volume) solution of methylcholanthrene in benzene reduced the total iron content of the epidermis of mice within a few days to approximately 50% of the normal content. Multiple applications of the carcinogen made on alternate days produced a further lowering. Ascorbic acid, on the other hand, was not significantly altered during the course of epidermal carcinogenesis.—F. L. H.

Reduction of Total Lipid-Protein Nitrogen Ratio of Mouse Epidermis by a Single Application of Methylcholanthrene. Wicks, L. F., and Suntzeff, V. [*Barnard Free Skin and Cancer Hosp., St. Louis, Mo.*] J. Nat. Cancer Inst., **3**:221-226. 1942.

The total lipid material extractable from the epidermis of mice was found to be much lowered a few days after even a single topical application of methylcholanthrene in benzene. Mice treated with benzene alone showed no such reduction. Protein nitrogen was used to indicate the amount of tissue involved.—F. L. H.

The Effect of Methylcholanthrene upon Epidermal Sodium and Calcium. Suntzeff, V., and Carruthers, C. [*Barnard Free Skin and Cancer Hosp., St. Louis, Mo.*] Cancer Research, **3**:431-433. 1943.

One application of methylcholanthrene reduced the calcium content of mouse epidermis within a few days to approximately 50% of the normal content. Multiple applications of the carcinogen on alternate days produced only slight further lowering. On the other hand, the epidermal sodium content was not significantly affected by similar treatment with the same carcinogen. Nucleoprotein phosphorus was used as a base of reference representing the amount of tissue involved. Three tables are appended.—Authors' abstract.

Unsaturated Fatty Acids in the Dietary Destruction of N,N-Dimethylaminoazobenzene (Butter Yellow) and in the Production of Anemia in Rats. György, P., Tomarelli, R., Ostergard, R. P., and Brown, J. B. [*Sch. of Med., Western Reserve Univ., Cleveland, and Ohio State Univ., Columbus, Ohio*] J. Exper. Med., **76**:413-420. 1942.

It has been observed that *N,N*-dimethylaminoazobenzene causes hepatoma in rats when incorporated in a diet of rice and olive oil but does not cause hepatoma in a diet containing casein, lard, sugar, cornstarch, salt mixture, cod liver oil, thiamin, riboflavin, pyridoxin, and pantothenic acid. However the latter diet became procarcinogenic when crisco or butterfat was substituted for lard and brown or white rice was substituted for the cornstarch and sugar. The authors assume that the diet became procarcinogenic, for these substitutions preserved the incorporated *N,N*-dimethylaminoazobenzene. It was found that unsaturated fatty acids, linoleic, arachidonic, and oleic acids, have a destructive effect on *N,N*-dimethylaminoazobenzene and that rice contains a stabilizer or antioxidant for this dye. Therefore, since replacement of lard in the diet by a more saturated fat, butter or crisco, decreases the rate of destruction of *N,N*-dimethylaminoazobenzene, and replacement of sugar and starch by rice prevents the destruction of the dye, the diet became procarcinogenic.—D. S.

Creatine and Creatinine Content of Transplanted Hepatomas and of Normal and Regenerating Liver. Greenstein, J. P. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:287-291. 1942.

The creatine and creatinine content of various normal and tumorous hepatic tissues was determined by the specific enzymatic method of Dubos and Miller. No difference was found in the true creatine content of transplanted rat hepatoma 31, of the livers of rats bearing transplanted hepatoma 31, of normal and of regenerating rat liver, all values being between 10 and 12 mgm. per 100 gm. of tissue. Similarly, transplanted mouse hepatomas 587 and 7A/77, the livers of mice bearing these tumors, and the livers of normal mice of the same strain showed no significant differences in creatine content. The values for the mouse tissues were 5 to 6 mgm. per 100 gm., or one-half those for the corresponding tissues of rats. The true creatinine content of all these tissues was vanishingly small. The total creatine chromogen of all the hepatic tissues of both rats and mice was 32 to 36 mgm. per 100 gm., and the total creatinine chromogen was 1.2 to 1.6 mgm per 100 gm. Thus, while a species difference was found in true creatine, none was found in total chromogen.—H. Q. W.

Demonstration of the Formation of Reticulin by Schwannian Tumor Cells in Vitro. Murray, M. R., and Stout, A. P. [*Coll. of Physicians and Surgeons, New York, N. Y.*] Am. J. Path., **18**:585-593. 1942.

A mediastinal neurilemmoma was successfully grown *in vitro*, and reticulin fibers were demonstrated in connection with the Schwann cells by means of Foot's silver impregnation method. The reticulin formation was slow, sparse, and sporadic in cultures of the neurilemmoma, and much less conspicuous than in cultures of the stroma of an adenomatous human parathyroid gland grown under comparable conditions. Six figures illustrate the Schwann cells growing in cultures and the association of reticulin therewith.—J. G. K.

Antigenic Relationship of Infectious Myxoma and Fibroma Viruses of the Rabbit. Shaffer, J. G. [*Johns Hopkins Univ., Baltimore, Md.*] Am. J. Hyg., **34-B**:102-120. 1941.

The myxoma and fibroma viruses appear to be basically related, since infection of the rabbits with one virus creates an immunity against the other. In the present work, an attempt was made to evaluate the antigenic relationship between the two viruses in terms of the complement fixation reaction. Specific complement-fixing antibodies against the myxoma and fibroma viruses were produced in sera of rabbits recovered from actual infection with the diseases or after injection of heat-inactivated virus suspensions. Stronger reactions were found when an antigen was tested against its own antiserum but cross-fixation reactions were obtained in all cases, indicating a close relationship between the virus antigens. The presence of soluble, closely related, heat-labile, complement-fixing bodies in myxoma and fibroma virus suspensions was demonstrated, in addition to a heat-stable complement-

fixing fraction in unfiltered viral suspensions. There is evidence that the presence of measurable fixing bodies in the serum is not necessary for immunity to myxoma. —A. C.

The Enduring Partnership of a Neoplastic Virus and Carcinoma Cells. Continued Increases of Virus in the V2 Carcinoma during Propagation in Virus-Immune Hosts. Kidd, J. G. [*Rockefeller Inst. for Med. Research, New York, N. Y.*] J. Exper. Med., **75**:7-20. 1942.

The V2 carcinoma, a transplanted rabbit cancer derived originally from a virus-induced papilloma and carrying in masked form the virus primarily responsible for it, was propagated in 5 successive groups of rabbits all previously hyperimmunized against the papilloma virus. It was found that the cancer grew as well in the hyperimmunized rabbits as in normal animals. In addition, serological tests showed that not only did the virus remain associated with the carcinoma cells but it also increased as the tumor grew in the hyperimmune animals. This latter result differed from the finding that during the propagation of the V2 carcinoma certain extraneous viruses were eliminated.—D. S.

Cell State as Affecting Susceptibility to a Virus. Enhanced Effectiveness of the Rabbit Papilloma Virus on Hyperplastic Epidermis. Friedewald, W. F. [*Rockefeller Inst. for Med. Research, New York, N. Y.*] J. Exper. Med., **75**:197-220. 1942.

It was found that a few preliminary applications to rabbit skin of tar, benzpyrene, or methylcholanthrene greatly enhanced the susceptibility of the skin to papilloma infection. However, these carcinogenic agents were employed for so short a time that they themselves elicited no tumors. Inoculation of the papilloma virus into skin that had been treated with these agents produced papillomas earlier and in greater numbers than on normal skin, and much higher dilutions gave rise to growths. This enhanced susceptibility was brought about by noncarcinogenic agents also; a mixture of turpentine and acetone was as effective as was tar or methylcholanthrene. The author suggests that this increased susceptibility of the altered skin may be due to an increased number of young susceptible cells and to a richer vascularization caused by the agents employed.—D. S.

Study of the Papilloma Virus Protein with the Electron Microscope. Sharp, D. G., Taylor, A. R., Beard, D., and Beard, J. W. [*Duke Univ. Sch. of Med., Durham, N. C.*] Proc. Soc. Exper. Biol. & Med., **50**:205-207. 1942.

Rabbit papilloma virus protein isolated in phosphate buffer by ultracentrifugation was photographed with the electron microscope at a magnification of 29,000 diameters.

The preparations of the papilloma virus protein appeared to be of considerable homogeneity with respect to particle size and shape. The mean diameter of the papilloma virus images was 44.0 mμ. They were circular in shape and usually single, although grouping occurred in preparations of virus concentrations higher than 0.1 mgm. per cc.—M. B.

Growth of Cancer Tissue in the Yolk Sac of the Chick Embryo. Taylor, A., Thacker, J., and Pennington, D. [*Univ. of Texas, Austin, Tex.*] Science, **96**:342-343. 1942.

Transplants of the mammary carcinoma of the dba and C3H strains were used. Tumor tissue, free of necrosis and infection, was squeezed through muslin. The resulting material was diluted with saline so that each milliliter of suspension contained about 0.2 gm. of tumor tissue. Fertile eggs incubated for 4 or 5 days at 38° C. were used for inoculation; 0.25 ml. of the tumor suspension was injected into the yolk, and the eggs were then incubated at 37° C. for 12 days or more. The injected tumor tissue became attached to the inner wall of the yolk sac from which it derived its blood supply. The bulk of the tumor grew down into the yolk. These tumors grew readily when transplanted back into mice. Tumor cells appeared also to be dispersed diffusely through the yolk substance. Histological sections showed healthy cancer cells with numerous mitoses. The supporting stroma was supplied by the yolk sac membrane.—M. B.

The Successful Production of a Mammalian Tumor with a Virus-Like Principle. Taylor, A. [*Univ. of Texas, Austin, Tex.*] Science, **97**:123. 1943.

Yolk sacs of chick embryos were implanted on the fifth day of incubation with saline suspensions of fresh tissue from a mammary carcinoma that had arisen spontaneously in a dba mouse. The eggs were then incubated for 12 days more, after which yolk was collected from those eggs bearing comparatively large tumors (1 gm. or more). The viscous yolk was diluted with an equal amount of saline solution, the material centrifuged, and the supernatant liquid passed through an N size Berkefeld filter. All operations were kept aseptic. Mice of the dba strain were injected subcutaneously with the filtrate (0.33 cc. per mouse).

Tumors developed at the site of injection, and smaller growths were found in the liver and in the visceral peritoneum of the digestive tract. The induced tumors were transplantable, grew rapidly, and histologically were composed of malignant cells of epithelial and connective tissue types.

The evidence indicates that the tumor cells constantly gave off the virus substance which was caught and preserved by the surrounding yolk. Whether the tumor factor is able to grow independently in the yolk medium has not been definitely determined.—M. B.

Effect of Climatic Environment upon the Genesis of Subcutaneous Tumors Induced by Methylcholanthrene and upon the Growth of a Transplantable Sarcoma in C3H Mice. Wallace, E. W., Wallace, H. M., and Mills, C. A. [*Coll. of Med., Univ. of Cincinnati, Cincinnati, Ohio*] J. Nat. Cancer Inst., **3**:99-110. 1942.

An experimental study of the effect of environmental temperature upon tumor genesis and growth. The study includes: (1) methylcholanthrene-induced tumors and (2) a transplantable tumor of induced origin. Induced tumors arose earlier in C3H mice kept at 92° F. and 50% relative humidity than in litter mates kept at 65° F. and variable humidity. When injected subcutaneously the transplantable sarcoma grew rapidly in animals in the hot room and slowly or actually regressed in those in the cold room. When injected intramuscularly the sarcoma grew equally well in both hot and cold room animals.—G. W. W.

Studies on the Effect of Hypothermia. I. Acute Physical and Physiological Changes Induced by the

Prolonged Hypothermic State in the Rabbit. Ariel, I., Bishop, F. W., and Warren, S. L. [*Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.*] CANCER RESEARCH, **3**: 448-453. 1943.

The body temperature of the rabbit can be reduced to very low levels by immersing the rabbit upright and chest high in ice and water. The rectal temperature can be lowered to 10° C. but the ability of the rabbit to recover spontaneously after it is removed to room temperature diminishes rapidly in the low body temperature ranges. Recovery is greatly accelerated by the application of external heat. A pseudohibernating state can be produced when the rectal temperature is lowered and maintained in the range between 23° and 28° C. Spontaneous recovery from this state can be brought about by exposing the animal to room temperature with the hair dry. The extent of the narcotic effect of the low temperature increases as the body temperature falls, producing at the low levels lack of movement and almost complete cessation of the cardiac and respiratory rhythm. Complete spontaneous recovery from this inactive state is rare but may be assisted by the application of external heat. Sudden death from convulsive seizures may occur at any stage in the reduction of the body temperature, but in the dormant state exitus may be so gradual that it cannot be well defined. It should not be regarded as final until all resuscitative efforts have failed. The reaction of the individual animal varies considerably in the time required to reduce the temperature, the lowest temperature level attained, the capacity for recovery, etc. The presence of a 2 week old Brown-Pearce epithelioma did not change the reaction of the animal to the hypothermia.—Authors' abstract.

Studies on the Effect of Hypothermia. II. The Active Role of the Thyroid Gland in Hypothermic States in the Rabbit. Ariel, I., and Warren, S. L. [*Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.*] CANCER RESEARCH, **3**:454-463. 1943.

When the body temperature of the rabbit is reduced to a level between 27° and 10° C. within a period of 2 to 5 hours, a gross enlargement of 2 to 3 times normal size is noted in the thyroid gland. There is microscopic evidence of vascular engorgement and increase in the size of the follicular cells. There is an accompanying tremendous increase in metabolism as the temperature starts to fall or during the recovery state from rectal temperatures near 25° C. As the body temperature falls, the metabolism diminishes rapidly, being nearly zero at the lowest rectal temperature measured (19° C.). There seems to be a direct correlation between the temperature level, the time and type of death, the duration of the low temperature state, etc., and the appearance of the thyroid cells. This is manifested by edema, apparent beginning disintegration of the nuclei, swelling of the cytoplasm, tremendous enlargement of the cells of the follicles, and disappearance of colloid. There is no evidence of multiplication of the cells, the main change seeming to be that of hyperactivity. Prolonged hypothermia and exhaustion states are accompanied by a picture resembling hypoactivity of the thyroid. In this case the follicular cells are thin and flat, and there is an excessive amount of colloid in the follicles. The ability of the rabbit to withstand the hypothermic state thus depends in part upon the state of the thyroid and its capacity to function over a prolonged period at low temperature.—Authors' abstract.

Studies on the Effect of Hypothermia. III. The Effect of a Single Short Period of Hypothermia on the Brown-Pearce Rabbit Epithelioma. Ariel, I., and Warren, S. L. [*Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.*] CANCER RESEARCH, **3**:464-470. 1943.

Short periods of low body temperature (6 hours at a rectal temperature of 18° C., 8 hours at 20° C., 24 hours at 30° C.) have a definite effect upon testicular transplants of the young Brown-Pearce rabbit epithelioma. An analysis of the averages of the growth curves indicates a definite retardation of growth during the first week, with restoration of the normal growth rate by the second week. The reaction of the individual tumor transplant may vary tremendously. Its growth rate may be increased, unchanged, or decreased. The occurrence of metastases seems to be definitely increased. In the gross and histologically, the tumor and the tumor site show congestion, edema, and focal hemorrhage with extensive diffuse necrosis. One week after treatment collars of rapidly growing tumor cells could be seen around the blood vessels. This offers an explanation for prompt recurrence. No definite "cures" were obtained by the experimental periods and technics used although a destructive effect was demonstrated.—Authors' abstract.

Studies on the Effect of Hypothermia. IV. The Rise of Serum Magnesium in Rabbits during the Hypothermic States as Shown by the Spectrochemical Method. Steadman, L. T., Ariel, I., and Warren, S. L. [*Univ. of Rochester Sch. of Med. and Dentistry, Rochester, N. Y.*] CANCER RESEARCH, **3**:471-474. 1943.

By the use of the spectrochemical method, it was found that the serum magnesium showed a rise of 24% above the control value after 2 to 5 hours in the hypothermic state. There did not seem to be any correlation between the serum magnesium levels and the extent or duration of the hypothermic state. There may be some relationship between the serum magnesium and the depressed thyroid activity, and the soporific state, corresponding to the findings in a normally hibernating animal. The serum sodium values fluctuated widely; no explanation can be given for this finding.—Authors' abstract.

Limits of Accuracy in Experimental Carcinogenesis as Exemplified by Tumor Induction with Ultraviolet Radiation. Blum, H. F., Grady, H. G., and Kirby-Smith, J. S. [*Nat. Cancer Inst., Bethesda, Md.*] J. NAT. CANCER INST., **3**:83-89. 1942.

The results of a considerable number of experiments on the induction of tumors by ultraviolet radiation are examined statistically to determine the accuracy to be expected in such studies. The logarithm of the induction time is normally distributed among individual male strain A albino mice. The distribution does not vary systematically with dosage of radiation, interval between exposures, intensity of the radiation, or age. The accuracy to be expected when experimental groups are compared is considered, and different methods of comparison are evaluated. The variation among mice is very wide, making it necessary to use large numbers of animals and to

give particular attention to methods of comparison if a reasonable degree of accuracy is to be achieved. Strictly, these findings apply only to induction of tumors by ultraviolet radiation, but it is probable that the wide variation found here is not unique in the field of cancer. If comparable latitude is found elsewhere, certain types of comparison in common use may prove unreliable.—Authors' summary.

Relationships between Dosage and Rate of Tumcr Induction by Ultraviolet Radiation. Blum, H. F., Grady, H. G., and Kirby-Smith, J. S. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:91-97. 1942.

The time required to induce tumors in 50% of a series of mice exposed to ultraviolet radiation was studied. The induction time was shorter when the same weekly dose was given in 5 or 7 exposures than when it was given in a single exposure. For a given schedule of exposures there was a minimum induction time that was not reduced by increasing the weekly dose, but below a certain dosage the induction time increased rapidly with decrease in dose. For a given dose the induction time was longer for older animals than for younger ones. It is pointed out that cells that are only slightly injured by ultraviolet radiation may recover between exposures and undergo no permanant change; cells severely injured may die and slough off. The cells receiving intermediate injuries are probably those which become cancerous. The authors are unable to describe the quantitative relationships found in terms of any one mechanism.—H. Q. W.

Carcinogenic Effectiveness of Ultraviolet Radiation of Wavelength 2537A. Blum, H. F., and Lippincott, S. W. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:211-216. 1942.

Considered in terms of surface dosage, wave length 2,537 Å is much less effective than longer wave lengths of the carcinogenic spectrum in inducing tumors and in producing other tissue changes. This is best explained by the fact that wave length 2,537 Å is absorbed principally in the stratum corneum before it reaches the viable cells. Thus it appears evident that photochemical changes taking place in the stratum corneum play no part in carcinogenesis and that the locus of carcinogenic action of ultraviolet radiation is the living cell itself.—F. L. H.

Types of Tumor Induced by Ultraviolet Radiation and Factors Influencing Their Relative Incidence. Grady, H. G., Blum, H. F., and Kirby-Smith, J. S. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**: 371-378. 1943.

The histopathology of tumors induced by a wide variety of dosages of ultraviolet radiation was studied. Spindle cell sarcomas arising from connective tissue elements predominated. Some polymorphous cell sarcomas occurred which possibly originated from muscle cells. Squamous carcinomas that arose in the epidermis or its appendages and that often occurred with sarcomas made up a small group of tumors. A few hemangioendotheliomas, one osteochondrosarcoma, and one sebaceous carcinoma were also found. Hemangioendotheliomas and spindle cell sarcomas of the eye are described. The authors conclude that ultraviolet radiation may induce tumors in a variety of tissues depending upon the susceptibilities of the latter and limited by the penetration of the radiation.

The ratio of carcinomas to sarcomas was not affected by dose *per se* but increased slightly with increase in tumor induction time. The ratio increased greatly with frequency of exposure, thus demonstrating a difference in response of epidermal and connective tissue elements.—F. L. H.

Carcinogenesis with Ultraviolet Radiation of Wave Length 2,800–3,400 Å. Bain, J. A., and Rusch, H. P. [*Univ. of Wisconsin, Madison, Wis.*] Cancer Research, **3**:425-430. 1943.

Tumors were produced by exposure of mice to the wave length band 2,800-3,400Å. A filter is described for the isolation of this band with the least possible loss of energy. Carcinogenesis could be effected with these wave lengths, but more energy was needed than when the whole mercury arc spectrum was employed, an implication that certain other portions of the spectrum can influence carcinogenesis. Small amounts of energy applied over a long period of time appeared to be more efficient for tumor production than large doses given during shorter periods. This indicated that there was a waste of energy when large doses were employed, or that the high levels had an additional retarding influence.—Authors' abstract.

P_{32} Uptake by Nuclei. Marshak, A. [*Univ. of California, Berkeley, Calif.*] J. Gen. Physiol., **25**:275-291. 1941.

A method is described for isolating nuclei in quantity from liver and tumor tissues with 5% citric acid. Radioactive phosphorus administered in the form of disodium phosphate was taken up rapidly by nuclei in the living animal, but not in tissue slices.

Nuclei of tumor cells accumulated more radioactive phosphorus than those of normal liver. This was shown to be due to mitotic activity and not to a form of metabolism peculiar to tumor cells.

As early as 1 hour after the administration of radioactive phosphorus, 60 to 70% of the total radioactivity of liver nuclei was found in the nucleoprotein fraction. This radioactivity was as great then as it was several days after injection, suggesting that conversion of phosphorus to nucleoprotein must be quite rapid. In rapidly growing lymphoma tissue the nucleoprotein fraction was found to contain 90 to 95% of the total nuclear radioactive phosphorus. From the rate of radioactive phosphorus uptake by nuclei it was calculated that a new lymphoma nucleus was synthesized on the average once every 27 hours.

Irradiation with 200 r x-rays altered the distribution of P^{32} in the lymphoma cell, increasing the concentration in the nucleus shortly after irradiation. The P^{32} concentration in the cytoplasm decreased with time after irradiation. The altered distribution may have resulted from the inhibition of mitosis produced by the x-rays.—J. L. M.

The Ultraviolet Spectrographic Examination of the Fat Fraction of Mouse Milk and Mammary Glands. DeOme, K. B., Strait, L. A., and McCawley, E. L. [*Univ. of California, San Francisco, Calif.*] Science, **96**:301-302. 1942.

If the mammary tumor-producing substance in the milk

of high tumor strain mice is similar to the estrogens or known synthetic carcinogenic hydrocarbons, it should be demonstrable by a comparison of the ultraviolet absorption spectra of the fat fractions of the milk and mammary glands of high and low tumor strain mice. Milk and tissue from nontumorous mammary glands from lactating high tumor strains A, C3H, and dba, and from the low tumor strain C57 black were studied. Freshly excised mammary glands were minced in a frozen state and extracted with acetone, ether, alcohol and isooctane. This procedure permitted study in the ultraviolet region down to 2,300 Å. The alcohol-soluble and alcohol-insoluble fractions were examined independently. Comparison of the ultraviolet absorption spectra of the milk and mammary gland tissue of these high and low tumor strain mice showed no significant differences. Ultra-centrifugation data recently published by other investigators have indicated that the active agent is primarily in the non-fat fraction.—M. B.

Technique Suitable for Quantitative Studies on the Mammary Tumor Inciter of Mice. Andervont, H. B., Shimkin, M. B., and Bryan, W. R. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:309-318. 1942.

Milk, pieces of spleen, and minced mammary gland from lactating strain C3H mice were administered to fostered strain C3H females. The oral administration of 0.1 to 0.8 cc. of milk to mice 7 to 10 days old produced mammary tumors. The subcutaneous injection of lactating mammary gland, splenic tissue, or 0.1 to 0.2 cc. of milk to mice 7 to 10 days old also produced tumors.

The subcutaneous injection of 0.15 to 0.25 cc. of milk to nursing mice produced tumors in 11 of 16 mice, whereas 0.5 cc. of milk to mice 30 to 57 days old produced tumors in 2 of 14 animals. The oral administration of 0.5 to 1.0 cc. of milk to mice 3.5 to 4 months old produced tumors in 3 of 24 mice. Older mice are apparently more resistant to the agent than are animals 7 to 10 days old.

Mice 7 to 14 days of age, derived from a cross of strain I females and C3H males, are suitable test animals for quantitative studies on the mammary tumor inciter of mice. They are highly susceptible to the agent and do not obtain it from their strain I mothers.—F. L. H.

Mammary Cancer in Fostered and Unfostered C3H Breeding Females and Their Hybrids. Bittner, J. J. [*Univ. of Minnesota Med. Sch., Minneapolis, Minn.*] Cancer Research, **3**:441-447. 1943.

As noted by Andervont, and as previously stated by the writer, the average age at which spontaneous mammary tumors appear in females of the C3H strain may decrease with continued inbreeding. The incidence of mammary tumors for the mice of groups with different average tumor ages remains the same when the incidence is based on animals living to the respective average tumor ages.

That foster nursing does not alter the inherited susceptibility for the development of spontaneous mammary cancer is demonstrated by the incidence of tumors appearing in the hybrids derived by mating unfostered (high tumor) and fostered (low tumor) C3H mice.

The active mammary tumor milk influence may appear at any time in the life of individual females. If the milk becomes active after the mice have given birth to young, the females usually die without cancer. The active milk influence is transferred to the progeny and the young that receive it usually die with cancer. This may indicate that the active mammary tumor milk influence must be present when the mammary glands start to develop if it is to play a role in the genesis of mammary cancer in mice.—Author's abstract.

Genetic Analysis of Susceptibility to Induced Pulmonary Tumors in Mice. Heston, W. E. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:69-78. 1942.

This is a genetic analysis in which susceptibility to induced pulmonary tumors is measured by the time at which the tumors arose and by the number of nodules found in each mouse. Crosses were made between strains A (highly susceptible) and L (highly insusceptible), and F_1, F_2, and backcross generations were raised. Intravenous injections of 0.5 mgm. of 1,2,5,6-dibenzanthracene dispersed in 0.5 cc. horse serum were given to mice of the 6 groups. The results confirm the conclusion that genetic as well as environmental factors are involved. It is also shown that genetic factors influencing susceptibility are multiple in number. A rough estimate shows that strains A and L differ by at least 4 pairs of factors and probably more.—G. W. W.

Inheritance of Susceptibility to Spontaneous Pulmonary Tumors in Mice. Heston, W. E. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:79-82. 1942.

Data on the incidence of spontaneous pulmonary tumors in mice at different age periods are presented. Mice of strain A (susceptible) and strain L (insusceptible to pulmonary tumor) were mated and the F_1 generation was produced as well as backcross hybrids resulting from mating the F_1 to the parent strain L. The data reported tend to run parallel to those published for induced pulmonary tumors. The results suggest that susceptibility to spontaneous pulmonary tumors in mice, like susceptibility to induced pulmonary tumors, is inherited on a multiple factor basis. It is pointed out that multiple factor inheritance offers an explanation for the variation in spontaneous pulmonary tumor incidence between the different inbred strains of mice.—G. W. W.

Genetics of the Susceptibility of Mice to a Transplantable Melanoma. Spangler, J. M., Murray, J. M., and Little, C. C. [*Univ. of Maine and Jackson Memorial Lab., Bar Harbor, Maine*] J. Nat. Cancer Inst., **3**:123-130. 1942.

A genetic investigation of the transplantable melanoma of the mouse, S91, with special reference to the F_2 albino hybrids of reciprocal crosses between mice of the A and dba strains is reported. The melanoma grows regularly and successfully in strain dba and fails to grow in the strain A mice. Results of transplantation into a large number of colored F_2 hybrids suggests that one mendelizing gene is involved in the growth of this tumor. Transplantation gives a different ratio with the albino F_2 animals. A tentative hypothesis which fits the experimental figures is advanced. It is suggested that further physiologic and genetic studies of melanotic tumors transplanted into albinos under various conditions of modification might well prove to be important.—G. W. W.

Relationship between the Lethal Yellow (A^y) Gene of the Mouse and Susceptibility to Induced Pulmonary Tumors. Heston, W. E. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:303-308. 1942.

The number of lung nodules per mouse, following intravenous injections of 20-methylcholanthrene dispersed in horse serum, was taken as a measure of susceptibility. It was found that yellow F_1 hybrid mice resulting from mating A strain females to Y strain males were more susceptible to induced pulmonary tumors than were their brown litter mates. Since the hybrid test animals can be considered isogenic except for this 1 pair of chromosomes, it is indicated that this increase in susceptibility is due to the A^y gene *per se*. It is suggested that the physiologic processes that are associated with the A^y gene and that lead to increased body weight of the yellow mice over their brown litter mates may also lead to the increased susceptibility to induced pulmonary tumors of the yellow mice.—G. W. W.

Milk Influence and Leukemia in Mice. Kirschbaum, A., and Strong, L. C. [*Univ. of Minnesota Med. Sch., Minneapolis, Minn., and Yale Univ. Sch. of Med., New Haven, Conn.*] Proc. Soc. Exper. Biol. & Med., **51**:404-406. 1942.

Reciprocal crosses to secure F_1 hybrids were made between strain F mice (high leukemia) and mice of 3 low leukemia strains (CBA, C57, and A). F_1 hybrids were backcrossed to the high (F) and low (A and CBA) leukemia strains in such a manner that strain F influence was supplied entirely by the male or entirely by the female parents in the 2 generations of the experiment. Observations were made also on F_2 hybrids where the female influence was provided by either high or low leukemia females. In addition, strain F mice were foster-nursed by animals of several low leukemia strains (CBA, I, A, C3H, NH). Transfer of the young to foster mothers was effected within 24 hours after birth.

Results from the breeding experiments listed above did not indicate that a specific milk influence (as for mammary cancer) is concerned in the development of leukemia in strain F mice. The incidence of leukemia in a group of 48 strain F mice fostered by low leukemia strain mice was, however, somewhat lower than in unfostered controls of the same strain.—M. B.

Induction of Leukemia in Mice by Methylcholanthrene and X-Rays. McEndy, D. P., Boon, M. C., and Furth, J. [*Cornell Univ. Med. Coll., New York, N. Y.*] J. Nat. Cancer Inst., **3**:227-247. 1942.

Leukemia was produced in mice by repeated percutaneous applications of methylcholanthrene. The animals were killed at intervals, blood-forming organs examined microscopically, and transmission experiments made with suspensions of cells from spleen and lymph nodes. Numerous transmission experiments made before the eighth week of painting were uniformly negative, although hyperplastic changes were evident in lymphoid tissues. Three cases of leukemia were first demonstrated by transmission experiments. A subsequent microscopic review showed the presence of collections of atypical cells in the splenic pulp and in lymph nodes, while the bone marrow and liver showed no change. The numerous transmission experiments made from histologically definite cases of leukemia failed in only 1 of 46 injected mice. Of the tests used to determine the presence of leukemia, gross examination is the least reliable, microscopic examination next in order, and inoculation experiments are the most decisive. The type of leukemia produced was lymphoid or atypical, with the exception of a few myeloid or monocytic leukemias. Evidence is presented suggesting that the atypical cells originate in lymphoid tissues. The onset of induced leukemia is sudden when judged by the criteria studied. The incidence of leukemia was slightly higher among the x-rayed and painted mice than among the mice painted but not x-rayed. A preliminary study of blood smears indicates that the majority of induced leukemias are accompanied by specific blood changes recognizable at least 2 weeks before the death of the animals.—Authors' summary.

Metabolism of Induced and Spontaneous Leukemias in Mice. Burk, D., Sprince, H., Spangler, J. M., Boon, M. C., and Furth, J. [*Nat. Cancer Inst., Bethesda, Md., and Cornell Univ. Med. Coll., New York, N. Y.*] J. Nat. Cancer Inst., **3**:249-275. 1942.

A study was made of the metabolism (Q values) and derived quotients of lymphoid, myeloid, monocytic, and atypical leukemias induced in mice by methylcholanthrene with or without x-irradiation. Lymph nodes, spleens, and livers were analyzed metabolically, examined histologically, and often tested for ability to transmit leukemia. In general, the results obtained with the two stocks of mice employed were similar, and no decided effects of sex, age, or x-irradiation were observed. The anaerobic glycolysis of lymph nodes and spleens with induced leukemias showed no definite metabolic distinctions ($>20\%$) between control, preleukemic, or leukemic mice, irrespective of type or extent of infiltration in the leukemic mice. The anaerobic glycolysis of leukemic livers, however, showed, depending upon the extent of infiltration, a twofold to eightfold increase over controls that took place simultaneously with histopathologic indication of onset of disease and successful transmission. All three types of analysis, metabolic, histopathologic, and transmission, concurred in indicating, after several months of painting, a sudden onset and rapid development involving but a few days.

The respiratory quotients of all tissues examined were well below unity, which is typical of both adult normal and malignant tissue. The oxidative metabolism (Q_{O_2} and Q_{CO_2}) of Rf lymph nodes and spleen showed a definite difference between normal and preleukemic organs on the one hand, and leukemic and leukemoid organs on the other, values for the latter groups being increased 25 to 75%. The aerobic glycolysis values ($Q_{O_{2A}}$) of preleukemic, leukemic, and leukemoid lymph nodes, spleens, and livers were 50 to 100% above normal. Chemical lactic acid determinations (Q_{LA}) agreed in general with the manometric glycolysis values (Q_A) under anaerobic conditions, but there were differences under aerobic conditions, especially in liver. No evidence was obtained for the existence of any preleukemic metabolism different from normal metabolism, except possibly for a small increase in aerobic glycolysis that developed some weeks

prior to the onset of leukemia. In general, the metabolism of the induced leukemias, while qualitatively characteristic of malignancy, quantitatively probably represents, together with certain chicken erythroleukoses, the lowest limit for any type of malignant tissue reported up to the present time.—Authors' abstract.

Studies on Fowl Leukosis. Transfer to the Chick Embryo. Pierce, M. [*Children's Mem. Hosp., Chicago, Ill.*] Am. J. Path., **18**:1127-1139. 1942.

Unfiltered suspensions of the spleen cells, leukocytes, and plasma of leukotic fowls induced leukosis in chick embryos when placed on the chorioallantoic membrane after the eighth day of incubation of the egg. Berkefeld filtrates of leukotic tissue and plasma failed to produce the disease in eggs similarly inoculated, though they gave rise to leukosis when injected intravenously into young fowls. The question whether the chorioallantoic membrane is unsuitable for growth of the virus is discussed. Seven illustrations.—J. G. K.

Histological Changes Preceding Spontaneous Lymphatic Leukemia in Mice. Potter, J. S., Victor, J., and Ward, E. N. [*Carnegie Inst. of Washington, Cold Spring Harbor, N. Y., and Coll. of Physicians and Surgeons, New York, N. Y.*] Am. J. Path., **19**:239-253. 1943.

A histological search was made for preleukemic changes in mice of the C58 strain, of which 90% die with some form of leukemia after the age of 6 months.

Of the C58 mice, 137, all clinically nonleukemic and aged between 176 and 382 days, were killed and the tissues examined: 59 of the animals exhibited reticulum cell hyperplasia, which was not seen in young C58 mice or in old mice of nonleukemic strains (total number of animals in the latter categories not given). In general the hyperplasia, which is described and illustrated in 7 figures, was confined to the reticulum of the medullary regions of lymph nodes or to the perivascular regions of the livers. The spleen seemed to be involved in only 3 animals. The distribution of the hyperplasia was limited, and in no case was there a generalized systemic reaction. When it occurred in lymph nodes, only 1 or 2 nodes were usually affected. In livers the lesions were not widely spread but were confined to a few foci.

In another group of 24 mice of strain C58, successive biopsies of lymph nodes were made at monthly intervals. Ten of the animals manifested leukemia at autopsy (eighth to twelfth months), which was preceded without exception by reticulum hyperplasia and lymphopoiesis, this appearing usually by the sixth month. In a further study a number of C58 mice were killed before and at 6 months of age and the tissues examined. Of the 24 animals less than 6 months old, none showed reticular hyperplasia, whereas of the 16 mice killed at 6 months, 7 showed reticular hyperplasia identical with that reported above in either lymph nodes or liver. Three Storrs-Little mice (a nonleukemic strain) showed no reticular hyperplasia when killed at 6 months.

The authors conclude that the restricted areas of reticulum hyperplasia in the lymph nodes and liver become the sites for primary malignant lymphocytopoiesis in C58 mice. Following the production of a population of free malignant lymphocytes, invasion accounts for the majority of the widespread lesions common to the terminal stages of leukemia.—J. G. K.

A Spontaneous Fibrosarcoma of the Foreleg and Paw in a C3H Mouse. Edwards, J. E., Dalton, A. J., White, J., and White, T. N. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:191-198. 1942.

The history and pathologic features of a spontaneous transplantable fibrosarcoma involving the foreleg and paw of a virgin female C3H mouse are reported.—F. L. H.

A Review of Some Spontaneous Neoplasms in Mice. Lippincott, S. W., Edwards, J. E., Grady, H. G., and Stewart, H. L. [*Nat. Cancer Inst., Bethesda, Md.*] J. Nat. Cancer Inst., **3**:199-210. 1942.

The article reviews the pathology of spontaneous neoplasms in mice with emphasis on the more common varieties such as those arising in the lung, mammary gland, liver, and the region of the salivary gland.—F. L. H.

The Occurrence and Transplantation of Embryonal Nephromas in the Rabbit. Greene, H. S. N. [*Rockefeller Inst., Princeton, N. J., and Yale Univ. Sch. of Med., New Haven, Conn.*] Cancer Research, **3**:434-440. 1943.

Observation of a large colony of rabbits over a period of 14 years disclosed 4 instances of embryonal nephromas. Study of the growths in both original and experimental hosts showed a morphological identity with similar human neoplasms, but because of the extreme variation in biological behavior in the two species it was concluded that the rabbit tumors are not analogous with the well defined clinical and pathological entity of Wilms' tumor in man.—Author's abstract.

Clinical and Pathological Reports

Diagnosis—General

An Evaluation of Peritoneoscopy. With Particular Reference to the Diagnosis of Abdominal Tumors. Garrey, W. E. [*Pratt Diagnostic Hosp. and Faulkner Hosp., Boston, Mass.*] New England J. Med., **225**:180-184. 1941.

The results of peritoneoscopy in 75 patients are reported. The gross pathologic lesions were identified and a correct diagnosis returned in 55 cases. In 25% of these, the findings fundamentally altered the treatment. The portion of the abdominal cavity seen by means of the peritoneoscope was found to be normal in 18 cases. Examination was unsatisfactory in 2 instances because of adhesions. The authors feel that peritoneoscopy is essential in order to avoid needless laparotomies.—G. H. H.

Skin and Subcutaneous Tissues

Melanoepithelioma (Melanosarcoma, Melanocarcinoma, Malignant Melanoma) of the Extremities. Bickel, W. H., Meyerding, H. W., and Broders, A. C. [*Mayo*

Clinic, Rochester, Minn.] Surg., Gynec. & Obst., **76**:570-576. 1943.

A review of 107 cases encountered during 24 years, with detailed reports of 4 illustrative cases. Of the patients treated by excision, 29.8% survived 5 years or more.—J. G. K.

Myxo-Sarcoma of the Skin. Maynard, M. T.-R. [*San Jose, Calif.*] California & West. Med., **57**:142-143. 1942.

A case report.—W. A. B.

Multiple Basal Cell Epitheliomas Originating from Congenital Pigmented Basal Cell Nevi. Nisbet, T. W. [*Pasadena, Calif.*] Arch. Dermat. & Syph., **47**:373-381. 1943.

A case report and review of the literature.—J. G. K.

Fibrosarcoma of the Soft Parts of the Extremities. Zieman, S. A. [*Rush Med. Coll., Chicago, Ill.*] Surgery, **9**:675-678. 1941.

A case report.—W. A. B.

Breast

Effect of Orchidectomy on Skeletal Metastases from Cancer of the Male Breast. Farrow, J. H., and Adair, F. E. [*Memorial Hosp., New York, N. Y.*] Science, **95**:654. 1942.

Bilateral orchiectomy was performed in a case of inoperable mammary cancer with osseous metastases. Four months later the breast ulcer had decreased in diameter, and there was complete relief from bone pain. X-ray studies indicated that the decalcified areas in ribs, vertebrae, and scapulae had not increased. Moreover increased density in these sites was considered a sign of increased calcification and healing.

The only chemical alteration observed was an increase of the serum alkaline phosphatase (5.9 to 11.1 units). A stable 17-ketosteroid output and a pronounced decrease in estrogen excretion were noted.—M. B.

Carcinoma of the Female Breast. Interval Report on the Results of Treatment. Graves, S. C. [*Free Hosp. for Women, Brookline, Mass.*] New England J. Med., **225**:57-61. 1941.

A report on 376 patients with carcinoma of the breast. Of these 36.7% were alive after 5 years. Radical mastectomy was performed in 284; in this group, 71% of the patients without axillary metastasis and 31% of those with axillary metastasis were alive after 5 years. Simple mastectomy, because of poor operative risk, was done in 38 cases with 62% 5 year survival. The total operative mortality was 1.3%. The author advises routine postoperative prophylactic x-radiation, especially in patients with tumors of high malignancy and no axillary involvement.—G. H. H.

Selection of Cases for Surgery in Cancer of the Breast. Kilgore, A. R. [*San Francisco, Calif.*] Radiology, **38**:540-542. 1942.

Radical surgery offers the best chance of cure in carcinoma still confined to the breast. Simple mastectomy is rarely justified. The selection of cases for primary surgical treatment should be further limited and those patients who show demonstrable neck or distant metastases, skin metastases, orange peel skin, fixation of the tumor to the chest wall, supraclavicular edema, or multiple large or fixed axillary nodes, should be treated radiologically.—C. E. D.

The Role of Estrogenic Substances in the Production of Malignant Mammary Lesions. Parsons, W. H., and McCall, E. F. [*Vicksburg Clinic, Vicksburg, Miss.*] Surgery, **9**:780-786. 1941.

Carcinoma of the breast (grade IV) developed in a 54 year old woman who had received approximately 200,000 units of theelin during 4 years.—W. A. B.

Management of Tumors of the Breast. With Special Emphasis on the Problem of Cancer of the Breast. Robillard, G. L., Auerbach, S. F., and Shapiro, A. L. [*Brooklyn Cancer Inst., Brooklyn, N. Y.*] Am. J. Surg., **60**:235-242. 1943.

A discussion of the management of 351 cases of carcinoma of breast.—W. A. B.

Sarcoma of the Breast. A Report of Twenty-Two Cases. Rogers, H., and Spencer, F. [*Harvard Med. Sch. and Massachusetts Gen. Hosp., Boston, Mass.*] New England J. Med., **226**:841-844. 1942.

Twenty-two cases of breast sarcoma are reported with a discussion of classification, treatment, and results. Of 18 patients (82%) whose tumors were classed as fibrosarcomas, 8 (44%) are known to be cured for at least 5 years, 6 are living under 5 years, and 4 are known to be dead of sarcoma. Of 4 patients whose tumors were classed as lymphoid sarcoma, angiosarcoma, or carcinosarcoma, all are known to be dead of sarcoma.

The treatment of choice is discussed. Regardless of the size and short duration of the tumor, the prognosis is poor unless the tumor is a fibrosarcoma.—C. W.

Cancer of the Breast. Results of Surgical Treatment at the Collis P. Huntington Memorial Hospital. Simmons, C. C. [*Massachusetts Gen. Hosp., Boston, Mass.*] New England J. Med., **226**:173-178. 1942.

A report and analysis of the results of surgical treatment of 135 cases.—C. W.

Significance of the Extent of Axillary Metastases in Carcinoma of the Female Breast. Warren, S., and Tompkins, V. N. [*Harvard Cancer Commission, Boston, Mass.*] Surg., Gynec. & Obst., **76**:327-330. 1943.

An analysis of 171 cases of breast cancer treated by radical mastectomy discloses that, as the extent of metastases to the axillary lymph nodes increases, curability as well as survival time of patients not cured decreases, and recurrence in the operative scar is more apt to occur.—H. G. W.

Female Genital Tract

Chorionepithelioma. A Study of Thirteen Cases. de Alvarez, R. R. [*Univ. of Michigan, Ann Arbor, Mich.*] Am. J. Obst. & Gynec., **43**:59-65. 1942.

The importance of the Aschheim-Zondek test in diagnosis is discussed. It is felt by the author that any patient showing a positive test 4 weeks after delivery following a full-term pregnancy or after an abortion, or in whom the test remains positive for 6 weeks or longer following the passage of a mole, presents strong evidence of an existing chorionepithelioma. It is emphasized that the

history, the microscopic appearance of the curettings, the Aschheim-Zondek test, and clinical judgment should be used collectively in arriving at a diagnosis. Total hysterectomy and bilateral salpingo-oophorectomy is considered to be the best form of treatment if the lesion is operable. Irradiation is considered of value only if the primary tumor has metastasized. Then the treatment is only palliative, with irradiation of the primary site and metastases. Where metastatic lesions are present, surgery is recommended only when continued and potentially fatal hemorrhage occurs. The urine of patients who have been operated upon should be checked at regular intervals. Routine x-ray and neurologic examinations prior to operation are recommended in all patients suspected of having chorionepithelioma.—A. K.

Carcinoma of Bartholin's Gland. Boughton, T. G. [*Lancaster Gen. Hosp., Lancaster, Pa.*] Am. J. Surg., **59**:585-591. 1943.

Case report.—H. G. W.

Pain Arising from the Female Pelvis: Differential Diagnosis and Treatment. Davis, M. E. [*Univ. of Chicago, Chicago, Ill.*] M. Clin. North America, **25**:35-53. 1941.

A general discussion on pain occurring in various pelvic conditions, including ovarian neoplasms and fibromyomas of the uterus.—J. L. M.

Myomectomy during Pregnancy. Hefferman, R. J. [*Tufts Coll. Med. Sch., Brookline, Mass.*] New England J. Med., **225**:185-187. 1941.

Four cases of myomectomy during pregnancy, with delivery at term, are presented.—G. H. H.

Tuberculoid Reaction in Ovarian Dysgerminoma. Heller, E. L. [*Butler County Mem. Hosp., Butler, Pa.*] Arch. Path., **35**:674-680. 1943.

Two cases of ovarian dysgerminoma accompanied by an unusual tuberculoid stromal reaction are reported. The epithelioid and giant cells are considered inflammatory in character rather than neoplastic.—J. G. K.

Granulosa and Theca Cell Tumors of the Ovary. With a Report of Thirty Cases. Henderson, D. N. [*Univ. of Toronto, Toronto, Canada*] Am. J. Obst. & Gynec., **43**:194-210. 1942.

Thirty ovarian tumors belonging to the granulosa and theca cell group of neoplasms are reported. From the standpoint of malignancy this group differs from other tumors of similar type reported in the literature. Of 14 cases that have been followed for 4 years or longer only one tumor proved to be malignant. Five instances of endometrial carcinoma and 10 of uterine fibroids were found in conjunction with these cases. The author feels that this suggests a direct relationship between the ovarian neoplasm and the uterine tumors but that no definite conclusion can be drawn.—A. K.

Association of Hydrothorax with Ovarian Fibroma (Meigs's Syndrome). Herrick, W. W., Tyson, T. L., and Watson, B. P. [*New York, N. Y.*] Arch. Int. Med., **71**:370-376. 1943.

A case report and a discussion of fifteen cases previously reported in the literature. The cause of the association of ovarian fibroma and hydrothorax remains unknown; removal of the fibroma effects permanent cure of the hydrothorax. There are 6 figures.—J. G. K.

Carcinoma of the Fallopian Tube. Kimball, T. S., Sanford, H. E., and Brown, A. F. [*Coll. of Med. Evangelists, Glendale, Calif.*] California & West. Med., **57**:351-353. 1942.

A report of 3 cases.—W. A. B.

Primary Carcinoma of the Fallopian Tube. McGoldrick, J. L., Strauss, H., and Rao, J. [*Brooklyn Cancer Inst. Brooklyn, N. Y.*] Am. J. Surg., **59**:555-562. 1943.

Report of 5 cases.—H. G. W.

Brenner Tumor of the Ovary Associated with Uterine Bleeding. Marwil, T. B., and Beaver, D. C. [*Woman's Hosp., Detroit, Mich.*] Am. J. Obst. & Gynec., **43**:99-104. 1942.

In this case a Brenner tumor seems to have secreted estrogenic hormone. The tumor occurred in a 77 year old woman, and many of the anatomic and physiologic changes usually associated with the presence of estrogens were found.—A. K.

Endocrine Effects of Certain Dysontogenetic Tumors of the Ovary. Novak, E. [*Baltimore, Md.*] Endocrinology, **30**:953-958. 1942.

Certain tumors of the ovary are made up of cells believed to date from the early stages of gonadogenesis. When the cells are dissociated from the normal course of differentiation at the undifferentiated phase of gonadal embryology, the resulting tumors have no endocrine potentialities and may occur in either the ovary (dysgerminoma) or testis (seminoma). When the tumors arise from certain elements persisting in the ovary and possess originally masculine potentialities, masculinizing tumors are produced (arrhenoblastoma), although it is not certain that other still poorly understood factors in sex differentiation may not play an important part in bringing about the sex character changes associated with such tumors. Finally, tumors arising from cells of the female mesenchyme, and assuming the form of either granulosa cell tumor or thecoma, possess definite feminizing effects dependent on their estrogenic function, and these manifestations depend much on the age at which such tumors occur. The incidence of arrhenoblastoma is far less than that of granulosa cell tumors or thecoma. The so called struma ovarii, or thyroid tissue tumor, also possesses endocrine capacities and is believed to have a teratomatous origin.—C. A. P.

Unique Cell Rest in a Uterine Fibroid. Peale, A. R., and Smith, L. W. [*Temple Univ. Hosp. and Sch. of Med., Philadelphia, Pa.*] Arch. Path., **35**:594-597. 1943.

A case is reported in which unidentified cell rests were found in what appeared to be a subserous uterine fibroid. Two figures are shown.—J. G. K.

Parvilocular Tumors of the Ovary. Schiller W. [*Cook County Hosp., Chicago, Ill.*] Arch. Path., **35**:391-413. 1943.

The parvilocular cystoma is an ovarian tumor characterized microscopically by small cystic cavities lined with a mucin-producing epithelium and embedded in fibrous stroma. Papillomatous proliferation and carcinomatous transformation may be observed. The parvilocular adenofibroma presents ducts and narrow glands embedded in a

well developed fibroma-like stroma; it probably originates from fetal remnants of the rete ovarii.—H. G. W.

Hydatidiform Mole and Chorionepithelioma. Schulze, M. [*Univ. of California, San Francisco, Calif.*] California & West. Med., **57**:292-294. 1942.

Observations on 16 cases of hydatidiform mole and 8 cases of chorionepithelioma with general discussion.—W. A. B.

Immature Botryoid Tumors of the Cervix. Simpson, E. E. [*Oroville, Calif.*] Arch. Path., **35**:535-545. 1943.

The paper presents a review of the literature, discussion, and report of a fatal case with metastases and widespread local extension. The primary tumor was composed of characteristic polypoid, grape-like bodies. Microscopic examination of tumor tissue removed from the cervix revealed round and spindle cells having frequent irregular mitoses, edematous mesenchymal stroma, myxomatous tissue, striated muscle cells, and a small area of early cartilage formation, suggesting that the growth may have been teratomatous. There are 5 figures.—J. G. K.

Carcinoma of the Endometrium. A Review with Results of Treatment through 1935. Smith, G. Van S. [*Harvard Med. Sch., Boston, Mass.*] New England J. Med., **225**:608-615. 1941.

A discussion of 307 consecutive cases. Of all treated patients, 58.1% were alive after 5 years, and of those treated before 1931, 45.4% were alive after 10 years. Treatment consisted of supravaginal hysterectomy, complete hysterectomy, radium therapy alone, and combined hysterectomy and radium or x-ray therapy. Statistically, the advantage of irradiation as a supplementary treatment to operation was questionable, but analysis of individual cases indicated that supplementary irradiation was of some value. The author noted that a high percentage of postmenopausal ovaries in patients with endometrial cancer showed thecal cell hyperplasia.—G. H. H.

Mesonephroma or Teratoid Adenocystoma of the Ovary. Stromme, W. B., and Traut, H. F. [*Cornell Univ. Med. Coll., New York, N. Y.*] Surg., Gynec. & Obst., **76**: 293-299. 1943.

A report of 10 patients, of whom 6 are dead or have extensive recurrence despite surgery and x-rays. The characteristic pseudoglomerulus is so infrequently demonstrable that the linkage with the mesonephros is not possible on the basis of present knowledge. The fact that these tumors secrete mucinous material and the finding of serous, granulosa, and thecal elements lead to the conclusion that for the time being it would be best to regard the group as teratoid cystadenomas.—H. G. W.

Five Tumors of the Round Ligament of the Uterus—One a Capillary Hemangioma. Schnedorf, J. G., and Orr, T. G. [*Univ. of Kansas Sch. of Med., Kansas City, Kans.*] Surgery, **10**:642-650. 1941.

Case reports concerning an endometrioma, a fibromyoma, a congenital multilocular cyst, a cystic hygroma, and a capillary hemangioma.—W. A. B.

Multiple Myomectomy. Sherwood, M. W., and Speed, T. [*Temple, Tex.*] Texas State J. Med., **38**:503-507. 1942.

The advisability of performing multiple myomectomy for uterine fibromyomas in women in the child-bearing age, rather than resorting to hysterectomy, is illustrated by the present report of 5 cases. Myomectomy may prove arduous, but favorable results can be achieved. The subserous, intramural, or submucous types of tumors can be excised, and as many as 14 individual growths were extirpated successfully from a single uterus. Three of the 5 patients bore children subsequent to myomectomy without difficulty. Symptomatically all 5 were cured. The number that remained fertile is in agreement with the percentages reported by other authors. In larger groups followed for longer periods of time, there is an expected recurrence of the uterine masses in approximately 5% of patients treated by myomectomy.—M. J. E.

The Treatment of Carcinoma of the Female Genitalia. Waugh, J. M. [*Mayo Clinic, Rochester, Minn.*] Proc. Mayo Clinic, **16**:94-95. 1941.

The treatment of the following tumors of the female genitalia are discussed: epithelioma of the vulva, adenocarcinoma of the small sebaceous cysts often seen about the vulva, primary carcinoma of the vagina, carcinoma of the uterine cervix, carcinoma of the uterine fundus, and tumors of the ovary.—J. L. M.

Male Genital Tract

Tumor of Spermatic Cord. Report of a Case. Marshall, V. F. [*Cornell Univ. Med. Coll., New York, N. Y.*] J. Urol., **48**:524-526. 1942.

Report of a tumor of the spermatic cord, composed of 3 closely related neoplasms; namely, a lipoma, a lipomyxoma, and a fibromyxolipoma.—H. G. W.

Prostatic Cancer: An Evaluation of Treatment by Castration. Rupel, E. [*Indiana Univ. Sch. of Med., Indianapolis, Ind.*] South, M. J., **36**:251-256. 1943.

A report of 26 cases.—W. A. B.

Lipomyxoma of the Spermatic Cord: Case Report and Review of Literature. Strong, G. H. [*Johns Hopkins Hosp., Baltimore, Md.*] J. Urol., **48**:527-532. 1942.

A lipomyxoma of the spermatic cord and 5 additional extratesticular scrotal tumors are recorded. Up to the present 257 tumors of the cord have been reported of which 64% were benign; the malignant growths were chiefly sarcomas.—H. G. W.

Primary Malignant Tumor of the Epididymis (Rhabdomyosarcoma). Strong, G. H. [*Johns Hopkins Hosp., Baltimore, Md.*] J. Urol., **48**:533-535. 1942.

The first recorded instance of a tumor of this type.—H. G. W.

Interstitial Cell Growths of the Testicle. Warren, S., and Olshausen, K. W. [*Harvard Cancer Commission, Boston, Mass.*] Am. J. Path., **19**:307-331. 1943.

The results of an extensive study of the interstitial cells of normal and diseased testes are presented as a basis for the differentiation between hyperplasia and neoplasia involving these cells. Two cases of hyperplasia and two of local tumor of interstitial cells are described, and the reported human cases of increase in number of testicular interstitial cells are reviewed. Of the 29 reported cases, 12 were classified as hyperplasia, 13 as local tumor, one as local tumor accompanied by hyperplasia, and 3 as malignant.—J. G. K.

Seminoma Developing in an Undeveloped Genital Anlage. Wells, H. G. [*Univ. of Chicago, Chicago, Ill.*] ARCH. PATH., **35**:590-593. 1943.

A case is reported of complete failure of the right genital anlage to develop, associated with immaturity of the left genital anlage. At the age of 47 the patient died with a retroperitoneal seminoma apparently arising in the rest of the undeveloped right genital anlage. Two figures are included.—J. G. K.

Pubertas Precox in a Six-Year-Old Boy Produced by a Tumor of the Testis, Probably of Interstitial Cell Origin. Werner, A. A., Spector, H. I., Vitt, A. E., Ross, W. L., and Anderson, W. A. D. [*St. Louis Univ. Sch. of Med., St. Louis, Mo.*] J. CLIN. ENDOCRINOL., **2**:527-530. 1942.

This is a detailed report of one of the rare cases of precocious development of male secondary sexual characters in a prepubertal boy as a result of a tumor of the testis. Pronounced growth of the external genitalia was first noticed when the boy was $5\frac{1}{2}$ years old. Before his seventh year the boy had genitalia described as adult in size; a deep voice; roentgenographic evidence of advanced osseous age; growth of hair over the pubis, axilla, upper lip, and face; moderate development of the prostate as determined by rectal palpation; well developed musculature; a height and weight above the average for a boy of his age. The Friedman test was negative. The urine was not assayed for follicle-stimulating hormone. Some evidence of libido was described.

The enlarged left testis was removed surgically and was found to have at one pole a firm yellow-gray circumscribed nodule which lay immediately beneath the capsule and was readily separable from the surrounding tissue. The cells of which the tumorous tissue was composed are described as morphologically similar to interstitial cells. Removal of the tumor was followed by regression in the development of secondary sex characters.—J. B. H.

Undescended Intra-Abdominal Testicle with Torsion of the Cord and Embryonic Cell Carcinoma. Case Report. Whittington, C. T. [*Piedmont Mem. Hosp., Greensboro, N. C.*] AM. J. SURG., **60**:304-305. 1943.

Case report.—W. A. B.

URINARY SYSTEM—MALE AND FEMALE

Bilateral Renal Carcinoma. Beilin, L. M., and Neiman, B. H. [*American Hosp., Chicago, Ill.*] J. UROL., **48**:575-584. 1942.

A ninth case of bilateral primary hypernephroid carcinoma is added to the literature.—H. G. W.

Certain Capsular and Subcapsular Mixed Tumors of the Kidney Herein Called "Capsuloma." Colvin, S. H., Jr. [*Mayo Clinic, Rochester, Minn.*] J. UROL., **48**:585-600. 1942.

According to the evidence gathered, it is believed that capsulomas are true tumors, some derived from the capsule, some from nephrogenic rests, and a few from the walls of the blood vessels. Histologically they contain 2 basic elements, smooth muscle and fibrous tissue, although a certain proportion contain fat and epithelial elements. Of 164 capsulomas reported in 144 cases among 2,634 consecutive autopsies, all were benign and were discovered incidentally at necropsy.—H. G. W.

Papillary Carcinoma of the Right Kidney. Report of a Case with Atypical History and Findings. Fowler, H. A. [*Washington, D. C.*] J. UROL., **48**:563-570. 1942.

Report of a case unusual in the extent of the metastasis. —H. G. W.

Myelocytoma of the Kidney: Report of a Case. Kretschmer, H. L. [*Presbyterian Hosp., Chicago, Ill.*] J. UROL., **48**:571-574. 1942.

Case report.—H. G. W.

Primary Papillary Carcinoma of the Ureter. With Report of a Case. Scholl, A. J., and Gallagher, V. J. [*Los Angeles, Calif.*] CALIFORNIA & WEST. MED., **57**:180-184. 1942.

Report of a case and a general discussion.—W. A. B.

Carcinoma of the Kidney and Pregnancy. Vitt, A. E., and Melick, W. F. [*St. Louis Univ. Sch. of Med., St. Louis, Mo.*] J. UROL., **48**:601-610. 1942.

A seventh case of hypernephroma of the kidney as a complication of pregnancy is added to the literature.—H. G. W.

Sarcoma of the Urinary Bladder. Wheelock, M. C. [*New England Deaconess Hosp., Boston, Mass.*] J. UROL., **48**: 628-634. 1942.

Four cases of sarcoma and one of malignant melanoma of the human urinary bladder are added to the literature. A case of myxosarcoma of the urinary bladder of a dog also is reported.—H. G. W.

Primary Carcinoma of the Urethra in the Male. Report of a Case. Zucker, M. O., and Weinstein, G. J. [*Lincoln Hosp., New York, N. Y.*] NEW ENGLAND J. MED., **225**:682-684. 1941.

A case report.—G. H. H.

LIVER

Benign Hepatoma: Review of the Literature and Report of a Case. Hoffman, H. S. [*George Washington Univ. Med. Sch., Washington, D. C.*] ANN. INT. MED., **17**: 130-139. 1942.

In the case reported, a tumor nodule 3 inches in diameter was removed from a liver otherwise apparently normal, although the adenoma exhibited cirrhosis.—H. G. W.

Large Solitary Bile-Cell Fibro-Adenoma of the Liver. Lee, E. S. PROC. ROY. SOC. MED., **36**:33-35. 1942.

A very large benign encapsulated tumor, composed of fibrous and myxomatous tissue and biliary epithelium, removed successfully from a boy of 13 months. No record of any similar case was found in the literature.—E. L. K.

Primary Carcinoma of the Liver. Cholangioma in Hepatolithiasis. Sanes, S., and MacCallum, J. D. [*Buffalo Gen. and Children's Hosps. and Univ. of Buffalo Med. Sch., Buffalo, N. Y.*] AM. J. PATH., **18**:675-687. 1942.

Two cases are reported of small, primary bile duct carcinomas (cholangiomas) of the liver associated with hepatolithiasis, cholangitis, and cholestasis. In one case papillomatous and adenomatous proliferation occurred

together with inflammation and lodgment of stones in bile ducts distant from the carcinomatous site. The authors consider both carcinomas directly related pathogenetically to the calculous cholangitis. Five figures.—J. G. K.

The Association of Primary Neoplasm of the Liver and Hemochromatosis. Saward, E. W. [*Peter Bent Brigham Hosp., Boston, Mass.*] New England J. Med., **226**: 264-266. 1942.

A case of hemochromatosis with primary hepatoma and metastasis to the brain, reported as an example of neoplasm associated with the cirrhosis of hemochromatosis.—C. W.

Muscle and Tendon

Synovial Sarcomesothelioma (Sarcoendothelioma). Fisher, H. R. [*Hahnemann Med. Coll. and Hosp., Philadelphia, Pa.*] Am. J. Path., **18**:529-553. 1942.

Forty-three cases collected from the literature and two new cases here reported comprise a small group of malignant tumors arising from synovial membranes. The tumors are composed of epithelial-like and sarcomatous cellular elements, both of which may be present in metastases. The origin and nature of the tumors are discussed.—J. G. K.

The Development of Sarcoma in Myositis Ossificans. Report of Three Cases. Pack, G. T., and Braund, R. R. [*Memorial Hosp., New York, N. Y.*] J.A.M.A., **119**: 776-779. 1942.

In the literature on myositis ossificans, reports were found of 5 cases in which the bony tissues involved in the disease underwent transformation into osteogenic sarcoma. The authors add 2 similar cases, and a third in which the ossifying lesion was associated with a malignant tumor, probably myxoliposarcoma.—H. G. W.

So-Called Myoblastoma. Report of Three Cases of Myoblastoma of the Skin and One Case of Myoblastoma of the Trapezius Muscle. Tuta, J. A., and Schmidt, F. R. [*Univ. of Illinois Coll. of Med., and Northwestern Univ. Med. Sch., Chicago, Ill.*] Arch. Dermat. & Syph., **46**:225-233. 1942.

A report of 3 cases of myoblastoma of the skin and one case of this tumor in the trapezius muscle. The nodules are found most frequently in the tongue; they are generally thought to be neoplastic and to be derived from primitive myoblasts. There is lack of agreement whether the nodules in striated muscle have the same origin as those, for example, found in the skin where there is no direct relation to striated muscle. The studies made by the authors indicate that the large cells of the nodules in the skin and in striated muscle are identical, but convincing proof of the myogenous nature of these peculiar nodules is lacking.—H. G. W.

Leukemia, Lymphosarcoma, Hodgkin's Disease

Aleukemic Leukemia. Fisher, H. R. [*Philadelphia, Pa.*] Pennsylvania M. J., **44**:1432-1439. 1941.

Many patients with leukemia, especially the acute lymphatic variety, present themselves in an aleukemic or subleukemic phase. The history and terminology of aleukemic leukemia are reviewed. The cases of this disease seen at the Hahnemann Hospital during the past 5 years are used to illustrate the various problems of diagnosis in the aleukemic phase. One case in particular exhibited an onset picture of aplastic anemia.—J. L. M.

Malignant Lymphoma. A Clinico-Pathologic Survey of 618 Cases. Gall, E. A., and Mallory, T. B. [*Massachusetts Gen. Hosp., Boston, Mass.*] Am. J. Path., **18**:381-429. 1942.

Finding it impracticable to classify malignant lymphomas on the basis of the distribution of the lesions, the authors have adopted a cytologic classification and have noted that the great majority of their cases could be readily divided into seven categories, as follows: stem cell lymphoma, clasmatocytic lymphoma, lymphoblastic lymphoma, lymphocytic lymphoma, Hodgkin's lymphoma, Hodgkin's sarcoma, and follicular lymphoma. This classification differs from previous ones primarily in the subdivision into two types of what has generally been grouped under the heading, reticulum cell sarcoma: one in which the cells are highly undifferentiated and resemble lymphoid stem cells, for which the name stem cell lymphoma is proposed, and a second in which the cells show recognizable features of differentiation in the direction of tissue phagocytes, which is termed clasmatocytic lymphoma. Repeated examinations showed the cytologic type usually to be remarkably constant in any one case, although a few instances were encountered in which there was progressive failure of differentiation as the disease progressed. By contrast, such features as the presence or absence of leukemia, generalization *versus* localization, and "sarcomatous" growth were found to be inconstant and changeable from time to time.

When the classification was put to the test of clinical correlation in 545 cases, sufficiently constant differences were found in age of onset, duration of the disease, frequency of involvement of various organs and tissues, tendency to localization or generalization, development of leukemia, and degree of radiosensitivity to delineate a series of recognizably different clinical syndromes. Follicular lymphoma was shown to be a form of malignant lymphoma and not, as has been reported, merely an inflammatory process.

On the basis of the recorded clinical and pathologic observations the authors conclude that prognostic implications may be guided to a surprising degree by the histologic character of the lesion. Unexpectedly prolonged survival periods were encountered, however, in cases in which the initial lesion appeared in the skin, bone, or viscera, and in those in which the primary lesion could be excised. Five year survivals occurred in all groups, ranging from 3% of patients with lymphoblastic lymphoma to 53% of those with follicular lymphoma.

Small doses of roentgen irradiation exerted a favorable effect, and there was evidence that such therapy prolongs life. Patients with clasmatocytic or stem cell lymphoma required larger dosages, and in a few instances, notably in lymphoblastic lymphoma and Hodgkin's sarcoma, irradiation was not beneficial.

Leukemia could not be predicted on the basis of any constant morphologic criterion. In fact, the blood picture often varied from time to time during the course of the disease, leukemic pictures alternating with nonleukemic ones, often quite irrespective of roentgen therapy. Hence the authors have come to regard lymphatic leukemia simply as a manifestation of an underlying lymphomatous process.

Twenty-one photomicrographs illustrate the various lymphomas.—J. G. K.

Chloroleukemia. Report of a Case with Special Reference to Its Neoplastic Nature. Hartz, P. H., and van der Sar, A. [*Pub. Health Service, Curaçao, N. W. I.*] Am. J. Path., **18**:715-727. 1942.

A case of chloroleukemia, occurring in a colored male 41 years old, is described. The leukemic cells were undifferentiated myelogenous elements, which invaded muscle fibers, blood vessels, lymphatics, and other tissues in the same manner as do tumor cells generally recognized as malignant. The relationship between the leukemias and the neoplasms is discussed briefly, and the similarity of the two processes emphasized.—J. G. K.

Leukemia: Agranulocytosis. Jackson, H., Jr. [*Harvard Med. Sch. Boston, Mass.*] New England J. Med., **225**: 978-982. 1941.

A review with emphasis on treatment.—G. H. H.

Insulin Resistance in a Case of Diabetes Mellitus and Chronic Lymphatic Leukemia. Report of a Case. Levi, J. E., and Friedman, H. T. [*Sinai Hosp., Baltimore, Md.*] New England J. Med., **225**:975-978. 1941.

Report of a case.—G. H. H.

Pituitary

Acromegaly: A Consideration of Its Course and Treatment. Report of Four Cases with Autopsies. Goldberg, M. B., and Lisser, H. [*Univ. of California Med. Sch., and Hosp. for Women and Children, San Francisco, Calif.*] J. Clin. Endocrinol., **2**:477-501. 1942.

This is a detailed consideration of the history and treatment of 4 acromegalics with postmortem studies. Emphasis is placed on therapeutic management based upon the endocrine rather than the strictly neurological status. Suggested criteria for following the changes produced by endocrine effects are serial determinations of: (1) the size of the head, chest, shoes, and gloves; (2) dental casts instead of x-ray films of the teeth; and (3) roentgenograms of the skull and sella turcica. Active progression of the disease is reported to occur even in the absence of such neurological signs as increased intracranial pressure, headache, visual disturbance, and characteristic limitation of visual fields. Evidence of progressive growth of the tumor is indicated by increase in the basal metabolic rate, lowering of carbohydrate tolerance, polydipsia, polyphagia, and excessive perspiration or attacks of sweating at night.

Irradiation of the pituitary gland is held to be the most effective form of therapy now available. With progression of the disease repeated courses of radiotherapy are advocated. The authors deplore a laissez-faire policy with regard to surgery in acromegalics whose life or vision is not threatened. Partial hypophysectomy is advocated, at least in patients who have failed to respond to irradiation of the pituitary region. The use of testosterone propionate in men and estradiol benzoate in women is considered a valuable adjuvant to other forms of therapy. —J. B. H.

Acromegaly with Long-Standing Tumor Infiltration of the Cavernous Sinuses. Spark, C., and Biller, S. B. [*Montefiore Hosp., New York, N. Y.*] Arch. Path., **35**: 93-111. 1943.

A case report.—H. G. W.

Thymus

Myasthenia Gravis Treated by Excision of the Thymic Tumor. Report of Two Cases. Campbell, E., Fadkin, N. F., and Lipetz, B. [*Albany Med. Coll., Albany, N. Y.*] Arch. Neurol. & Psychiat., **47**:645-661. 1942.

Of 7 patients with myasthenia gravis studied at the Albany Hospital in the past 6 years, roentgenographic evidence of mediastinal tumor was obtained in 3. The paper reports the cases of 2 patients in whom the thymoma was removed surgically. One patient was considerably improved, although mild myasthenic symptoms persist after 2 years. Death of the other patient, 2 days after operation, was attributed partly to bronchopneumonia.

The view is discussed that benign tumor and hyperplasia of the adult thymus are in some way associated with myasthenia gravis. Present knowledge is insufficient to establish whether this relation is primary or secondary.—A. C.

Removal of Malignant Thymoma in a Case of Myasthenia Gravis. Turnbull, F. [*Vancouver Gen. Hosp., Vancouver, Canada*] Arch. Neurol. & Psychiat., **48**:938-945. 1942.

Successful removal of the thymic tumor failed to relieve the symptoms of myasthenia gravis.—A. C.

Thyroid

Parathyroid Adenoma. Report of Three Cases. Cochrane, R. C. [*Harvard Med. Sch. and Boston City Hosp., Boston, Mass.*] New England J. Med., **224**:973-978. 1941.

Three cases are reported. In two, the tumor was found in an unusual location: one tumor was within the body of the thyroid gland, the other within the thorax. In the third case, in which an uneventful convalescence occurred, partial, rather than complete removal of a large adenoma was performed.—G. H. H.

A Severe Case of Parathyroid Adenoma. Curtis, C. S., and Loomis, E. G. [*St. Anthony Hosp., St. Anthony, Newfoundland*] New England J. Med., **225**:370-371. 1941.

Report of a case with multiple pathological fractures. The patient recovered following excision of the tumor. —G. H. H.

The Mouth in Hyperparathyroidism. Strock, M. S. [*Harvard Dental Sch., Boston, Mass.*] New England J. Med., **224**:1019-1923. 1941.

A discussion of dental and jaw abnormalities encountered in hyperparathyroidism.—G. H. H.

Multiple Tumors

Multiple Carcinoma. The Clinical Picture, Diagnosis and Prognosis. Hellandall, H. [*Coll. of Physicians and Surgeons, New York, N. Y.*] Am. J. Surg., **60**:22-35. 1943.

An analysis of 30 cases of multiple cancers.—W. A. B.

Multiple Primary Neoplasm of the Colon. Herrlin, J., Jr., and Mersheimer, W. L. [*N. Y. Med. Coll., Flower and Fifth Avenue Hosp., New York, N. Y.*] Am. J. Surg., **60**:126-129. 1943.

A case report.—W. A. B.

Multiple Primary Tumors of the Spinal Cord. Lichtenstein, B. W., [*Univ. of Illinois, Coll. of Med., Chicago, Ill.*] Arch. Neurol. & Psychiat., **46**:59-71. 1941.

A report of two cases of multiple intramedullary tumors (ependymomas). Multiple neurinomas arising from the spinal nerve roots and a dural meningioma in the thoracic region were also present in one of the cases. The clinical and pathological aspects of the findings are discussed.—A. C.

Granulosa Cell Tumor of the Ovary and Coincident Carcinoma of the Uterus. Stohr, G. [*Woman's Hosp., New York, N. Y.*] Am. J. Obst. & Gynec., **43**:586-599. 1942.

Three cases of granulosa cell tumor of the ovary are described in which there is said to be "glandular hyperplasia of the endometrium attaining the character of neoplastic growth productive of a variety of malignant features." The author feels that these cases might be comparable to those in mice where carcinoma of the uterus has been produced by administration of large qauntities of estrogenic hormone; in the author's patients the granulosa cell tumors were the site of production of estrogenic substance. In one instance the uterine growth is said to have regressed following removal of the ovarian tumor. This is considered to be in conformity with the animal experiments in which the endometrial glandular epithelium "may be restored to normal when stimulation by the hormone ceases."—A. K.

Miscellaneous

Aspiration Biopsy, with a Description of a New Type of Needle. Franseen, C. C. [*Huntington Memorial Hosp., Palmer Memorial Hosp., and Massachusetts Gen. Hosp., Boston, Mass.*] New England J. Med., **224**:1054-1058. 1941.

A needle employing the principle of the trephine is described.—G. H. H.

Latent Primary Cancer. Gewanter, A. P., Mitchell, N., and Angrist, A. [*Queens Gen. Hosp., Jamaica, N. Y.*] Arch. Path., **35**:66-84. 1943.

The term "latency" is used in cancer which produces symptomatic precocious metastases while itself remaining silent. In a series of 2,514 necropsies, 25 cases of carcinoma meeting this definition were encountered, constituting 5.9% of all cases of carcinoma.—H. G. W.

Delay in the Treatment of Cancer. Harms, C. R., Plaut, J. A., and Oughterson, A. W. [*Yale Univ. Sch. of Med., New Haven, Conn.*] J.A.M.A., **121**:335-338. 1943.

Delay by the patient was defined as failure to consult a physician although symptoms had persisted for one month or longer. Delay by the physician referred to any period of waiting over the 3 weeks allowed for diagnosis. A study of 158 successive cancer patients admitted to the clinic revealed that in 54.8% of the cases, the patient was responsible for delay, in 17.4%, the physician, and in 27.8%, both the patient and the physician. The most common cause for delay by the patient was the idea that the symptoms were not serious enough to warrant medical care.—H. G. W.

Recent Advances in the Study of Cancer. Horsley, J. S. [*St. Elizabeth's Hosp., Richmond, Va.*] South. M. J., **36**: 8-12. 1943.

A general discussion, with advocacy of castration in cancer of the prostate and mammary gland.—H. G. W.

Melanoma. A Review of Thirty-Two Cases Admitted to the Brooklyn Cancer Institute during a Five-Year Period. Howes, W. E., and Birnkraut, M. [*Brooklyn Cancer Inst., Brooklyn, N. Y.*] Am. J. Surg., **60**: 182-189. 1943.

A review of 32 cases.—W. A. B.

Colloid Adenocarcinoma of the Urachus. Report of Two Cases. Hughes, P. B., and La Towsky, L. W. [*Univ. of Pennsylvania Hosp., Philadelphia, Pa.*] Am. J. Surg., **58**:422-425. 1942.

A report of 2 cases.—H. G. W.

The Estimation of Operative Risk in Patients with Cancer. Johnson, A. S., and Lombard, H. L. [*Westfield State Sanatorium and Pondville Hosp., Massachusetts Dept. of Pub. Health, Mass.*] New England J. Med., **224**:759-762. 1941.

A study of case records of the Westfield State Sanatorium and of Pondville Hospital showed that the operative mortality in patients with cancer was determined for the most part by the age of the patient and the length of the operation. The influence of such factors as obesity, malnutrition, and hypertension was of relatively little importance.—G. H. H.

Papillary Cystadenoma Lymphomatosum. Lederer, M., and Grayzel, D. M. [*Jewish Hosp., Brooklyn, N. Y.*] Arch. Path., **34**:833-842. 1942.

Four cases are reported with a review of the literature. The evidence at hand indicates that the tumor arises from heterotopic salivary gland epithelium enclosed in lymphoid tissue.—H. G. W.

Mesothelioma (Endothelioma) of the Peritoneum. Ramsey, T. L., and Chomet, B. [*St. Vincent's Hosp., Toledo, Ohio*] Arch. Path., **35**:292-298. 1942.

Report of a case.—H. G. W.